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COTTON WILT: A PATHOLOGICAL AND PHYSIOLOGICAL INVESTIGATION[1]

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INTRODUCTION

Cotton wilt is undoubtedly the most important disease of the cotton crop in the United States and causes the largest annual loss. According to records of the Plant Disease Survey of the U. S. Department of Agriculture compiled for several years, the disease is widely distributed through the cotton belt from Virginia to Texas, and is apparently spreading from year to year to new localities.

The damage caused by this important disease of cotton varies in different localities each season, but frequently large areas are involved and, without question, the annual loss to the entire cotton belt must reach into the millions of dollars. The estimated reduction in yield in the United States because of wilt was 350,000 bales in 1925. In Mississippi, one of the leading cotton-producing states of the mid-south, the annual loss from this disease is fully 50,000 bales, and it reaches even larger proportions during unusually dry seasons.

In view of the wide prevalence of wilt in Mississippi and other southern states, an investigation of this problem was begun by the writer at the Missouri Botanical Garden in the fall of 1925, where a year was spent in a laboratory and greenhouse investi-

[1] An investigation carried out at the Missouri Botanical Garden in the Graduate Laboratory of the Henry Shaw School of Botany of Washington University and submitted as a thesis in partial fulfillment of the requirements for the degree of doctor of philosophy in the Henry Shaw School of Botany of Washington University.

gation of this disease and of the causal organism. The study of the problem has been continued since this time at the Mississippi Agricultural Experiment Station. Briefly, the objects of the investigations reported in this paper are: (1) to ascertain some of the physiological relations of the fungus causing the disease, such as growth-temperature relations in pure culture, effects of H-ion concentration upon growth, and the factors involved in producing wilt by the fungus; (2) to determine the pathogenicity of the fungus; (3) to determine the effects of varying conditions of nutrition upon the development of the disease under greenhouse and field conditions; (4) to study differences in varietal susceptibility and resistance in both upland and staple cottons.

PREVIOUS INVESTIGATIONS ON COTTON WILT

One of the first investigators to establish the identity and probable relationship of the fungus associated with cotton wilt was Atkinson ('92). He considered the wilting of the plant to be due to the plugging of the vessels of the plant near the base of the stem by the mycelium of a fungus. He made isolations from the tissues of affected plants and described the fungus found as a new species under the name *Fusarium vasinfectum* Atk. Atkinson also found a species of *Fusarium* on the surface of some of the infected plants, but considered this a saprophyte, distinct from the internal fungus. Some years later, Smith ('99) made a more extensive study of the Fusaria found associated with wilt in cotton, in cowpea, and in watermelon. This investigator was under the impression that he had developed the ascigerous stage of the fungus on cotton, and this he designated *Neocosmospora vasinfecta* (Atk.) Smith. He stated that the fungus probably also occurred on okra, but that its parasitism and the genetic connection of the various spore forms were not proved and that chlamydospores were not observed. At the time these investigations were made, Smith seems to have had no doubt as to the parasitic nature of the cotton or cowpea wilt fungus, or as to the genetic relationship of the various spore forms found on cotton. He admitted that these points had not been definitely settled through satisfactory infection experiments, by deriving one spore form from the other in pure cultures. The field evidence, how-

ever, of the parasitic nature of the cotton and cowpea fungus was thought by Smith to be most convincing, since the fungus was always present in the vessels of the diseased plants and the disease occurred year after year on the same soils. More recent studies by Higgins ('09) and Butler ('10) indicate that in all probability there is no genetic relationship between the *Neocosmospora* referred to by Smith and the internal fungus *Fusarium vasinfectum* which Atkinson associated with cotton wilt. Neither did Higgins regard the *Neocosmospora* as having any connection with the wilt of cowpea. In fact, a study of the literature indicates that with cultures of *Fusarium vasinfectum* Atk., isolated from wilted plants and accordingly capable of infecting cotton, perithecia have never been produced. It appears, therefore, that the correct identity of the fungus associated with the wilt disease of cotton should properly date back to Atkinson's work in Alabama in 1892.

Orton ('00) apparently accepted Smith's determination for cotton wilt as *Neocosmospora vasinfecta* and was under the impression that the wilt of okra was caused by the same fungus. He states: "No inoculation experiments have been tried, but in the experience of the writer, okra has never failed to contract the disease when planted in fields infected with the cotton wilt disease."

Orton ('00) studied the period of incubation, or the interval elapsing between the time the young seedlings of cotton were exposed to the attacks of the fungus and the first visible external symptoms. He found that a time interval of forty days or longer was required. He noted the conditions which favored the progress of the fungus through the plant and although these were not fully understood, he states: "From observations that have been made, it is believed that highly fertilized plants, growing vigorously, succumb more readily than those which have grown on poorer soil." Orton also corroborated the work of Smith ('99) and Atkinson ('92) by producing the disease in cotton plants through infection experiments. This was accomplished by inoculating the soil, in which cotton was growing, with pure cultures of *Fusarium vasinfectum*. He conducted experiments with a number of fungicides, spraying these on the plants and

applying them to the soil in the hope of killing the fungus, but none of the results obtained gave promise of combating the disease successfully. The fungicides used were Bordeaux mixture, Bordeaux mixture and sulphur, copper carbonate, copper acetate, lime, sulphur, lime-sulphur mixture, liver of sulphur, iron sulphate, carbolic acid, caustic soda, formalin, and kainit. Fields were selected which were uniformly infested, but the cotton died in both the treated and untreated areas, and no difference traceable to the fungicides used could be observed.

At that time, Orton advocated such hygienic treatment as rotation of crops and the removal of diseased plants, also the avoidance of spread of the fungus by cattle, tools, or through infested manure. Considerable stress was also placed upon developing wilt-resistant selections of cotton. Fulton ('07), working in Louisiana in 1907, advocated the planting of wilt-resistant varieties of cotton and the employment of crop rotation as a method of control.

In 1908, Orton published a popular account of cotton wilt in which the geographical distribution of the disease, amount of loss, symptoms of wilt, and the life history of the fungus, so far as known at the time, were given. The influence of seasonal conditions, the time of planting, soil conditions, and the effect of fertilizers and fungicides upon wilt were noted. Attention was also given to breeding cotton for wilt-resistance, and breeding methods were described.

Lewis ('11) noted the distribution of wilt in Georgia in 1911 and estimated the annual loss in that state to be about three-quarters of a million dollars. He studied the effect of fertilizers on the disease and concluded that various combinations of acid phosphate, muriate of potash, and guano,[1] used at the rate of from 300 to 800 pounds per acre, were of no benefit in controlling it. The test plats were not replicated, however, and the size of the plats in the experiments was not indicated.

Lewis ('15) originated several wilt-resistant varieties of cotton from 1905 to 1915, some of which are still widely planted in the southeastern section of the cotton belt. He used both the method of selection and of hybridization of desirable parent

[1] Mixed fertilizer, 10–2–2.

plants to obtain resistant strains. Doubtless his better-known wilt-resistant cottons are Lewis 63, Dix-afifi, and Council Toole.

Rast ('22) conducted some fertilizer experiments in Arkansas in 1920 on the control of cotton wilt, which apparently were beneficial. A portion of a field was observed by him in 1920 in both fertilized and unfertilized areas heavily infested with wilt. The grower had used 500 pounds per acre of a mixture containing 10 per cent phosphoric acid, 3 per cent nitrogen, and no potash. So many plants in this field on both the fertilized and unfertilized areas died in 1920 that no record was kept of the yield of cotton. The experiment had been planned to run on this area for 5 years, and it was necessary to change radically the plan of the experiment in order to justify the continuance of the work. It was suggested that 500 pounds of the same fertilizer that was used in 1920 (containing 10 per cent phosphoric acid, 3 per cent nitrogen, and no potash) be mixed with 500 pounds of kainit containing 12.5 per cent potash. This mixture contained, as shown by analysis, 5 per cent available phosphoric acid, 1.5 per cent nitrogen, and 6.25 per cent potash. It was applied in the spring of 1921 at the rate of 1000 pounds per acre before planting. His results are quoted: "The plants on the unfertilized area began to die long before they were mature and were evidently infected with the wilt disease. By harvest time, no less than 95 per cent of the plants on the unfertilized area were dead and not a dead plant could be found in any part of the field where the fertilizer was used. The fertilized area produced 1127 pounds of seed cotton per acre; whereas the disease-infested part of the field to which no fertilizer was applied produced only 225 pounds of seed cotton per acre. Plants on an adjoining area to which 500 pounds per acre of fertilizer containing 10 per cent available phosphoric acid, 3 per cent nitrogen, and no potash were applied before planting and to which 500 pounds per acre of kainit were used as an additional application after the plants were up and growing, were equally resistant to the disease and just as prolific." Rast concluded that the above results were due to the potash in the fertilizer, and Elliott (Rast, '22, p. 224) who examined the plats, wrote as follows: "I do not attempt to make an explanation of the facts, but to all appearances the treatment the kainit plat

received enabled the plants this year to very largely escape wilt infection."

DESCRIPTION OF COTTON WILT

A noticeable yellowing of the leaves of the cotton plant accompanied by a stunted appearance somewhat early in the season is usually a good indication of *Fusarium* wilt. The disease may be noted at first in irregular spots over the field, and each succeeding year these infested areas enlarge. Frequently the main stem of plants infected with the disease remains short, while some of the lower branches grow normally (pl. 31, fig. 1). It is, however, by means of the internal appearance of affected cotton plants that we obtain the most characteristic symptom of wilt. If the stem of a freshly wilted plant is cut across near the ground and a brown or black discoloration of the fibro-vascular tissues is found, there is strong evidence of the disease (pl. 31, fig. 2). The tap-root of infected plants is shorter and the lateral roots less abundant. If plants are examined late in the season at the time they are approaching maturity, the wilt fungus may frequently be observed not only in the lateral and tap-roots, but in the vascular tissues of the main branches, the leaf petioles, and pedicels.

MORPHOLOGICAL AND CULTURAL CHARACTERS OF THE FUNGUS

The fungus, *Fusarium vasinfectum* Atk., the pathogen responsible for the occurrence of cotton wilt, has not been made the object of extensive morphological or taxonomic study by the writer. It seems desirable, therefore, to accept the criteria of taxonomic differentiation of species as established through the work of Wollenweber and associates ('25). The fungus is accordingly placed in section Elegans of the genus *Fusarium*. Growth of the fungus is usually rapid on a variety of complete nutrient media, and both micro- and macro-conidia are produced readily in culture. Micro-conidia are present on the aerial mycelium and are dominantly non-septate, ovoid to fusoid, 4–6 × 3–4.5 μ. Macro-conidia are usually none to 3-septate, attenuate at distal end, pedicellate, fusoid to sickle-shaped, 20–25 × 4–6 μ. Chlamydospores are produced in cultures 3 weeks old or less and may be either terminal or intercalary (pl. 32,

figs. 3–4). The color of the stroma is marked, and on sterilized rice the acid modification is especially well developed, the rose-red to wine-red color appearing in this medium in from 8 to 12 days after the fungus is introduced. The aromatic odor of the fungus when grown on rice is pronounced. On nutrient and bean agar the color of the mycelium is white, but on starchy media, such as potato-dextrose and corn-meal agar, the mycelium is tinged noticeably purple.

SOURCES OF CULTURES

Several strains of *Fusarium vasinfectum* have been used during the progress of this investigation. On September 15, 1925, the writer isolated the fungus from the stem tissues of a Cook Triumph hybrid cotton plant growing in a breeding plot of the Experiment Station, A. & M. College, Mississippi, and later established the pathogenicity of this culture by a series of infection experiments. This isolation has been used very largely in the earlier phases of the investigation and particularly in the laboratory studies of the fungus. On July 15, 1926, the fungus was isolated from a wilted plant of Trice cotton in the breeding plot at the Experiment Station, A. & M. College. It was also isolated from a wilted plant of Delfos 6102 cotton collected at Dunleith, Mississippi, on August 16, 1926, and from Trice cotton growing at Poplarville, Mississippi, on August 12, 1926. Strains of the fungus supplied by Dr. V. H. Young and Dr. H. R. Rosen, of the Arkansas Experiment Station, have been included in some of the experiments. The pathogenicity of all cultures was later established by infection experiments.

The method of isolation may be briefly described as follows: Cotton plants showing unmistakable symptoms of wilt were collected and free-hand sections made of the stem tissue and occasionally the leaf petioles. The sections with typical vascular discoloration were treated from 3 to 5 minutes in a 1 : 1000 solution of mercuric chloride (holding with sterile forceps), rinsed in sterile distilled water, and placed on nutrient, bean, and potato-dextrose agar in Petri dishes. As a disinfecting agent in the preparation of material, calcium hypochlorite has also been used extensively in making isolations, and is equally satisfactory for the purpose.

PHYSIOLOGICAL STUDIES OF THE FUNGUS

INFLUENCE OF TEMPERATURE

Temperature relations of many organisms causing plant diseases are important in that the geographical distribution of these diseases may be influenced very largely by their thermal relations. For instance, it is a well-known fact that many of the Fusarium diseases develop in destructive form only during periods of prolonged hot weather when soil temperatures are high, whereas others may require moderately cool temperatures to attain the maximum degree of development necessary for infection.

A very large amount of work has been reported by various investigators on temperature relationships of many fungi causing plant diseases, and particularly is this true of the Fusarium group of diseases. It is impossible to go extensively into the literature on this subject in the scope of this paper, but the work of a few investigators may be noted:

Gilman ('16) found that *Fusarium conglutinans* Wollenw., the fungus causing cabbage yellows, has a high optimum temperature and is very resistant to drying both in pure culture and in the soil. The occurrence and development of the characteristic symptoms of the disease required a temperature of about 17–22° C. or higher. Lower temperatures (12–16° C.) under controlled conditions prevented the occurrence of the trouble in the greenhouse. Field observations made over a period of years bore out this relation between the occurrence of the disease and high temperature.

Edgerton ('20) has worked on the temperature relations of tomato wilt, and he and other investigators state that the tomato wilt fungus, *Fusarium Lycopersici* Sacc., is more commonly found in the warmer regions throughout the world. He finds that this fungus grows best at a temperature around 29° C. and that infection also takes place quicker and the disease develops in plants more rapidly if the soil is kept around that temperature. Very little development of the disease is noted at lower temperatures.

Tisdale ('23) found that cabbage yellows is often most destructive in Wisconsin during midsummer when the soil is dry and hot. On the other hand, there is little or no development of the disease, even on soils heavily infested with the organism,

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during moist, cool weather. This author emphasizes the importance of the temperature relation of this fungus in the geographical distribution of the disease. For instance, in the southern states, where cabbage is generally grown commercially as a winter or early spring crop, the soil temperature is very probably too low for the organism to gain a foothold on such plants. However, when once introduced, the *Fusarium* establishes itself on summer-grown cabbage and other related hosts and attacks the crop whenever soil temperatures are favorable.

Clayton ('23) found somewhat similar temperature relations with the tomato-wilt fungus as did Edgerton ('20), but noted that the most favorable temperatures of soil and air, as determined in tanks in the greenhouse, are 27° C. and 28° C. respectively, i.e., after the fungus was established in the stems of the plants.

Jones and Tisdale ('22) found that in the case of flax wilt caused by *Fusarium Lini* Bolley, the disease was aggravated by hot seasons and that the midsummer temperature in the regions where it occurred corresponded closely with the optimum for the fungus.

In view of the rather extensive investigations which have been made on the *Fusarium* wilts of other crops, it was interesting to study some of the temperature relations of the cotton-wilt fungus, *Fusarium vasinfectum*. Moreover, it has been noted by the writer not only in Mississippi, but in Alabama, Louisiana, Arkansas, and Texas, that wilt of cotton is usually more prevalent during prolonged periods of dry, hot weather. Particularly was this true in the Gulf Coastal States during the years of 1924–25, two seasons which were notable because of extended periods of dry weather and abnormally high temperature during the greater part of the summer.

In studying the effects of temperature upon the development of the fungus in pure culture, uniform Petri dishes containing a measured amount (10 cc.) of potato-dextrose agar were used. In order to obtain spores of the fungus free of mycelium, a suspension of the spores was made in test-tubes containing 3 or 4 cc. of sterile distilled water. The spore suspension was made from a 7-day-old culture of the fungus growing on steamed rice. In one Petri dish, a thin layer of nutrient agar was poured and then

several loopfuls of the spore suspension were placed at random over the surface of the plate. As soon as the agar solidified, the plates were examined by the low power of the microscope, and single spores were located. These areas were immediately marked by means of a blue wax pencil and the agar blocked out by means of a needle and transferred to the center of the Petri dishes. Each temperature series was run in triplicate and measurements of the growth of the colonies of the mycelium were made at the end of 2, 6, 10, and 15 days. The results are given in table I.

TABLE I

GROWTH OF FUSARIUM VASINFECTUM AT VARIOUS TEMPERATURES

Temperature degrees C.	Diameter of colony in cm. at indicated intervals											
	2 days			6 days			10 days			15 days		
	Plate No.			Plate No.			Plate No.			Plate No.		
	1	2	3	1	2	3	1	2	3	1	2	3
6	0	0	0	0.2	0.2	0.2	1.2	1.5	1.2	1.5	1.6	1.6
10	0.4	0.3	0.2	1.8	1.9	1.8	2.5	2.8	2.9	3.8	3.6	3.4
15	1.9	1.8	1.8	3.8	3.7	3.9	5.4	5.2	5.4	8.2	8.0	8.1
20	3.1	3.2	3.1	7.0	7.2	7.0	9.0*	9.0*	9.0*	9.0*	9.0*	
24	3.8	3.7	4.0	7.8	7.5	8.0	9.0*	9.0*	9.0*	—	—	—
28	4.1	4.0	4.5	7.9	8.0	8.2	9.0*	9.0*	9.0*	9.0*	9.0*	†
30	3.8	3.7	4.0	7.7	7.5	8.0	9.0*	9.0*	9.0*	9.0*	9.0*	—
35	1.8	1.4	1.6	3.2	3.3	3.6	3.5	3.0	3.5	—	—	—
40	0.0	0.0	0.0	0.0	0.0							

* Growth over plate.
† Contaminated.

It will be noted from the above results that *Fusarium vasinfectum* grows very slowly at the lower temperatures, and that the optimum is not far from 28° C. The optimum temperature, therefore, of the parasites of the very similar Fusarium diseases of cotton, tomato, flax, and cabbage is practically the same in pure culture, although the host plants have quite different temperature relations. This fact is supplemented by the work of Edgerton ('20), Tisdale ('23), and others. The maximum temperature of *Fusarium vasinfectum* is apparently around 38° C.

The effect of temperature on the growth of *Fusarium vasinfectum* was also studied by means of liquid cultures. The dry weight of the fungous mat was ascertained at various tempera-

tures ranging from 6 to 40° C. at intervals of 5, 10, and 15 days. Duggar's[1] solution was used for growing the fungus and the final concentration of chemicals used in this solution was as follows: M/4 dextrose, M/5 KNO_3, M/20 KH_2PO_4, M/100 $MgSO_4$, and a trace of $FePO_4$. For obtaining these dilutions the following stock solutions were prepared: M/2 dextrose, M/1 KNO_3, M/4 KH_2PO_4, M/10 $MgSO_4$, and M/1000 $FePO_4$. For 50 cc. of medium the following amounts of these solutions were used: 25 cc. dextrose, 10 cc. KNO_3, 10 cc. KH_2PO_4, 5 cc. $MgSO_4$, and 6 drops $FePO_4$. Pyrex flasks were used for the cultures, and these were run in triplicate at each temperature. A copper-lined, differential incubator with compartments was used in these experiments. It was heavily insulated with asbestos, and had an ice chest at one end and on the opposite end a compartment for water was heated by a small gas burner regulated by a thermostat. With such radiation almost any temperature could be maintained by simply increasing or diminishing the size of individual compartments opposite the ice chest and water-heated unit. The flasks were inoculated with a 7-day-old culture of the fungus by means of a sterile needle in the usual manner, and care was taken to introduce approximately the same amount of the inoculum into each flask. At the intervals indicated, the mats were collected on a filter paper by means of a Buchner filter, dried to constant weight in a Fries oven, and weighed.

Using the dry weight of the fungous mat as an index, it will be

TABLE II

GROWTH OF FUSARIUM VASINFECTUM AT VARIOUS TEMPERATURES

Temperature degrees C.	Dry weight of fungous mat in milligrams		
	5 days	10 days	15 days
6	6.20	54.9	81.4
10	28.0	84.5	97.1
15	35.8	98.7	145.2
20	38.2	149.4	251.5
25	49.5	112.3	455.2
28	226.0	338.6	610.0
30	382.3	493.7	725.2
35	286.5	294.2	422.9
38	172.0	247.3	*

* Contaminated.

[1] A nutrient solution for fungi recommended by Dr. B. M. Duggar.

noted again that the optimum temperature for *Fusarium vasinfectum* is around 28 and 30° C. It grows slowly at temperatures below 10° C.; the growth curve begins to fall sharply at about 30° C., while at 38° C. it falls off abruptly. The results are graphically illustrated in fig. 1.

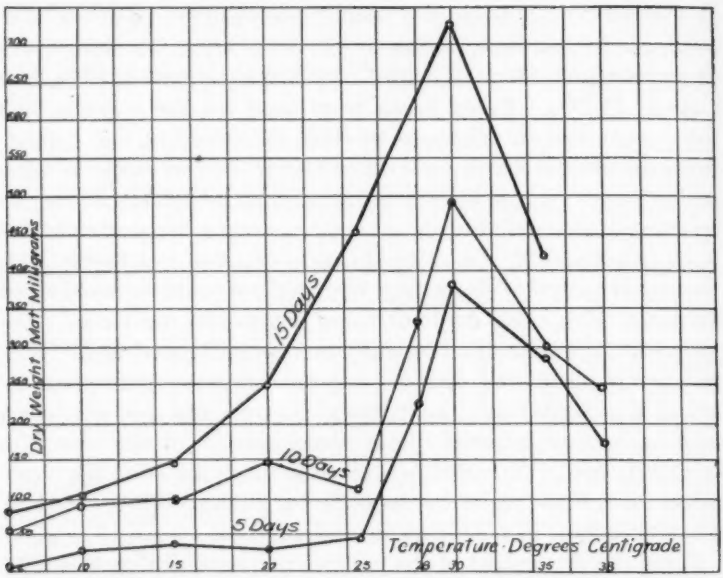

Fig. 1. Showing the effect of temperature on the growth of *Fusarium vasinfectum*.

RELATION TO HYDROGEN-ION CONCENTRATION

The effect of the hydrogen- and the hydroxyl-ion concentration upon the germination of the spores and growth of various fungi has been demonstrated by numerous investigators. That the reaction of the substratum is a very important factor in influencing the growth of many disease-producing organisms is a well-known fact. The literature bearing upon this subject is rather extensive, and has been reviewed to advantage by Webb ('19, '21), Lehman ('23), and others. Webb ('21) finds that with a certain *Fusarium* sp., germination is equally as good, if not better, with an alkaline reaction. However, with the majority of fungi he

employed the maximum for germination was between P_H3.0 and 4.0.

Hopkins ('21), using a synthetic medium in which the hydrogen-ion concentration was adjusted by means of KH_2PO_4, K_2HPO_4, H_3PO_4, and KOH, found that a *Fusarium* isolated from scabby wheat, but not proved to be *Gibberella*, exhibited a similar depression in the growth-acidity curve as did *Gibberella saubinettii*, the wheat-scab organism. In the case of the latter, the amount of growth increased with decreasing acidity from P_H2.5 to a maximum of P_H 4.0–4.5. It then decreased to a minimum at P_H 5.0–5.5 and rose again to a second maximum, but the highest point was not determined.

MacInnes ('22) observed that a strain of *Fusarium* isolated from scabby wheat in Minnesota was capable of growing in solutions varying through an unusually wide range of hydrogen-ion concentrations. The organism grew in nutrient media ranging from P_H 5.0 to P_H 11.7. No determinations were recorded for hydrogen-ion concentrations on either side of these values.

Kirby ('22) found that the maximum hydrogen-ion concentration for *Fusarium moniliforme* on corn was near 8.2, and the range of growth was greater than P_H 5.2–9.2.

The results of the above-mentioned investigations and many others clearly indicate that many fungi require varying degrees of acidity in the substratum for optimum growth, whereas others flourish to better advantage in an alkaline medium or in one approaching the neutral point. In the case of the organism causing the "take-all disease" of wheat, Kirby ('22) states that the fungus requires alkalinity for optimum growth, and that may explain why additions of alkaline substances to the soil have been reported as favoring the fungus, while additions of acid-forming substances decrease the amount of infection. From the work of numerous investigators, notably Martin ('20, '21), Lint ('14, '15, '16), and others, it is known that there is a correlation between increase in soil acidity and a decrease in the prevalence of the potato-scab organism, *Actinomyces scabies*. According to Chupp ('25) and others, a neutral soil with P_H value of 7 will almost invariably give a crop 100 per cent scabby, while an acid one with a P_H value of 5.6 or greater will produce an almost clean crop.

In order to gain an idea of the limiting hydrogen-ion concentration for the growth of *Fusarium vasinfectum* some growth tests were made. Duggar's solution previously referred to was used and contained inorganic salts and dextrose in the following volume molecular concentration: M/4 dextrose, M/5 KNO_3, M/20 KH_2PO_4, and M/100 $MgSO_4$. Six drops of M/1000 $FePO_4$ were used for each 50 cc. of solution, and M/5 $Ca(NO_3)_2$ was substituted for the M/5 KNO_3. The inorganic salts were Merck's "blue label" reagent grade, and the dextrose was the grade designated as "difco standardized." The nutrients were prepared in 500-cc. Pyrex flasks in quantities of 475 cc. for each series, and this quantity of solution was adjusted to the desired P_H value by the addition of previously determined amounts of 0.1647 *N* H_3PO_4 or 0.1613 *N* KOH. The range of initial P_H values was from 2.5 to 9.0 After P_H adjustments were made, the 400 cc. of nutrient solution were divided as follows: 50 cc. of the solution were pipetted into each of eight 125-cc. Pyrex flasks so as to have each series in duplicate for examination after certain growth periods. The remaining 75 cc. of solution of each initial P_H value were put into Jena test-tubes and sterilized for 30 minutes at 15 pounds pressure in order to note the effect of sterilization upon P_H and to serve as controls for final P_H reading at the end of designated growth periods. It was found that autoclaving did not change the P_H appreciably in the tubes, but to guard against enolization of sugars in the alkaline ranges, it was deemed best to sterilize the dextrose portion separately for each flask. This was added later to the solution of salts by means of a sterile pipette.

The cultures were inoculated with a spore suspension of *Fusarium vasinfectum* in sterile distilled water, one loopful being added to each flask, and placed in the incubator at a temperature of 29° C. The cultures were also wrapped with carbon paper to exclude the light from the bulbs. Two flask cultures from each series were examined at the end of 8, 12, 16, and 28 days, and the dry weight of mat, hydrogen-ion concentration, and titrable acidity were determined at each examination. The P_H values were determined colorimetrically by the method of Clark and Lubs ('17).

By means of a Buchner suction filter, the mats were collected on filter-paper, the dry weight of which had been previously determined. The mats were dried for 3 days in a Fries oven at 100° C., cooled in a calcium chloride desiccator for a uniform period, and weighed. The filtrate from each mat was diluted to 100 cc. with distilled water and the total acidity was determined by titrating a 10-cc. aliquot of the filtrate with N/50 KOH, using phenolphthalein as an indicator. The amount of unused sugar present at each interval was also determined for series 2, 7, and 12, and for these determinations the method of Shaffer and Hartman ('21) was employed. The data obtained are shown in table III.

TABLE III

GROWTH OF FUSARIUM VASINFECTUM IN NUTRIENT SOLUTION*

Series No.	Initial P_H	Growth period days	Dry wt. mat mgms.	Final P_H	Cc. N/50 KOH to neutralize 10 cc. filtrate	Wt. of sugar per cc. of solution mgms.
1	2.5	8	49.0	2.9	19.45	
		12	121.3	2.9	18.07	
		16	387.4	3.0	19.00	
		28	348.4	6.6	11.2	
2	2.8	8	49.6	3.2	19.9	43.2
		12	145.0	3.4	17.6	24.0
		16	385.0	3.4	16.5	2.24
		28	397.0	6.2	11.9	0
3	3.0	8	51.2	3.5	16.3	
		12	301.0	5.5	13.4	
		16	414.0	5.8	13.1	
		28	756.3	6.4	5.4	
4	3.5	8	112.2	4.6	16.6	
		12	343.5	5.7	14.2	
		16	417.1	5.8	13.6	
		28	761.0	6.9	1.2	
5	4.0	8	116.0	4.5	16.8	
		12	345.2	5.8	13.4	
		16	419.0	5.8	14.2	
		28	758.3	7.3	.8	
6	4.5	8	122.3	5.3	14.9	
		12	357.2	6.6	10.7	
		16	418.2	6.9	3.9	
		28	747.0	7.4	1.9	
7	5.5	8	133	5.3	15.2	28.08
		12	362.3	6.6	9.8	4.8
		16	413.0	6.8	7.1	2.88
		28	732.6	7.2	2.3	0

TABLE III (Continued)

Series No.	Initial P_H	Growth period days	Dry wt. mat mgms.	Final P_H	Cc. N/50 KOH to neutralize 10 cc. filtrate	Wt. of sugar per cc. of solution mgms.
8	6.0	8	195.0	5.6	14.7	
		12	380.0	6.4	5.9	
		16	441.6	6.4	6.1	
		28	577.8	7.3	3.7	
9	6.5	8	199.0	6.2	10.6	
		12	353.0	6.6	9.9	
		16	434.1	7.2	3.9	
		28	452.0	7.4	1.3	
10	7.0	8	189	6.4	4.3	
		12	318	6.4	4.35	
		16	447	6.2	8.6	
		28	512	7.2	1.75	
11	7.5	8	144	6.4	3.4	
		12	358	6.2	9.6	
		16	455	6.3	9.4	
		28	502	7.4	0.30	
12	8.5	8	109	6.9	3.8	30.2
		12	299	7.0	.4	22.2
		16	421	7.6	.55‡	9.8
		28	529	8.4	1.3‡	0
13	8.8	8	87	6.7	5.2	
		12	257	6.9	2.7	
		16	405	†—	——	
		28	487	8.5	1.2‡	
14	9.0	8	96	8.4	.95‡	
		12	251	7.9	.70‡	
		16†	——	——	——	
		28	427	7.8	.85‡	

* The values given are the averages of 2 cultures.
† Contaminated.
‡ .2 *N* HCl.

The relations of time to growth in the series are shown in the curves in fig. 2. At the end of the 28-day period, the best growth, as indicated by the dry weight of mat, occurred in series 3–8 inclusive, represented by P_H values of 3.0, 4.0, 4.5, and 5.5. The maximum growth occurred in the cultures started at P_H 3.5. The growth of the fungus was slow in series 1, 2, and 3 for the first 8 days, but later the growth rose rapidly and reached a maximum at the end of 28 days as high as other series started with higher P_H exponents. Judging from the behavior of the cultures in series 1 and 2, the fungus tolerates a culture solution

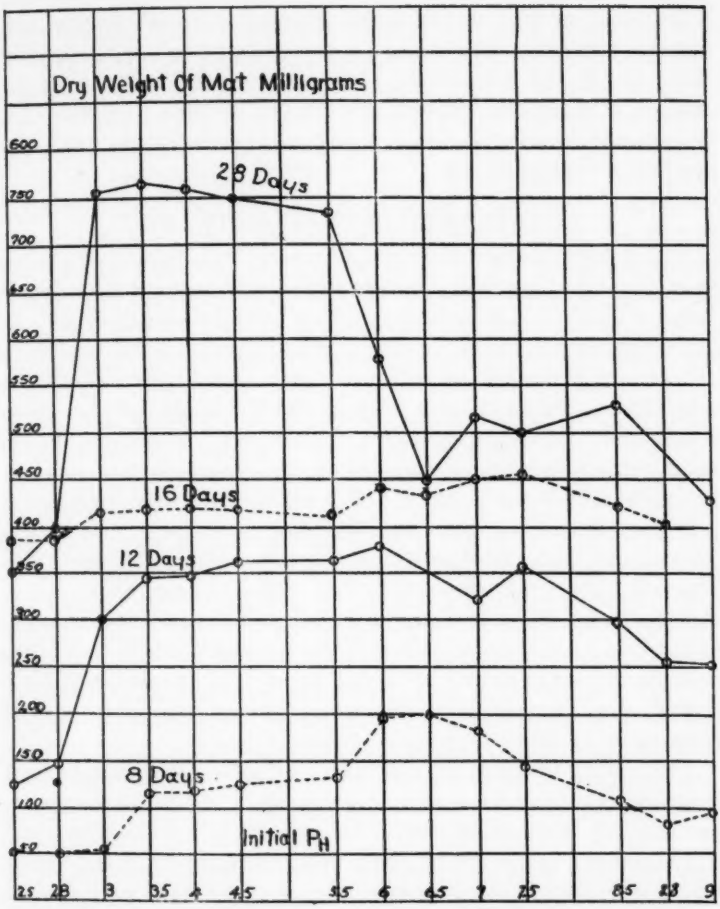

Fig. 2. Showing the relation of time to the growth of *Fusarium vasinfectum*.

strongly acid. However, the maximum growth attained at the close of the experiment was notably less with the more acid cultures. All series initially acid became less so, while all initially alkaline became slightly acid excepting a few started on the extreme alkaline side of P_H 7, which, although becoming acid at first, reversed the reaction later and were distinctly alkaline at the close of the growth period.

In the majority of the series changes in total acidity were closely correlated with changes in hydrogen-ion concentration. Decrease in the amount of sugar in the culture was also correlated with an increase in dry weight of mat. At the close of the experiment (28 days) sugar had entirely disappeared from the culture solution.

FACTORS INVOLVED IN PRODUCING WILT

The wilting in the case of many Fusarium diseases of plants has been explained by numerous plant pathologists and botanists as being due to mechanical plugging up of the vascular system by the vegetative growth of the fungus. Smith ('99) suggested this theory in the case of cotton, cowpea, and watermelon plants, which were infected with closely related fungi of the genus *Fusarium*. He found considerable mycelium within the water-conducting tubes, and assumed that the amount present was sufficient to act as an obstruction of liquid upward, thus causing the condition known as wilt. Among others who have held similar views in the past in connection with cotton wilt are Duggar ('09), Stevens ('21), and Orton ('00, '08, '12). It may be stated in fairness to the above investigators that in the case of the majority of the Fusarium diseases, this idea was generally held by botanists and plant pathologists until a few years ago; the writer is fully aware, too, that some of the investigators mentioned above, perhaps all of them, no longer hold such views.

The theory that the wilting may be due to simply mechanical plugging of the water-conducting vessels of the plant, however, has recently been questioned by a number of investigators, and the numerous experiments along this line have been concerned with a number of Fusarium wilt diseases of plants of very diverse habitat. Because of the fact that the writer has frequently observed considerable fluctuation in the prevalence of cotton wilt in fields which were known to be infested, and the fact that the disease has appeared to be more severe during dry seasons, especially in recent years, it was of interest to make some studies regarding the phenomena concerned in wilting by *Fusarium vasinfectum*.

If the cause of wilting was simply a response to mechanical

obstruction, it is conceivable that with ample moisture, there would perhaps be a greater vegetative growth of the fungus within the tissues and a corresponding increase in wilt because of greater obstruction by the mycelium. In this connection, observations made in Mississippi by the writer are somewhat in disagreement with the statement by Elliott ('21). He remarks: "It is a well observed fact that cotton wilt is more severe in a wet season than in a drier one. The reason for this is because the weather conditions which favor a rapid 'sappy' growth of the cotton plant also favor the development of the wilt fungus, and if the water supply becomes at all short, such plants suffer quickly and severely." Observations made in Mississippi and other states lead the writer to believe that the reverse may be true; i. e., the disease is certainly more serious during prolonged periods of abnormally dry weather, but may frequently develop suddenly and in destructive form as soon as such periods are broken by ample rainfall. Beal ('26) has also noted a greater prevalence of the disease during periods of dry weather. Therefore, since a study of the relation of moisture conditions to the occurrence of wilt did not strengthen the theory that the wilt may develop as the result of mechanical plugging of the vessels owing to increased growth of the fungus, it was desirable to study other factors which might be involved. This appeared essential, too, since microscopic examination of the vascular system of many cotton plants with typical wilt symptoms failed in many instances to reveal the presence of hyphal filaments in the vessels, and in others, when the fungus was detected, it was only in very meager amounts.

Some of the experiments of other investigators on the factors involved in wilting of plants by other Fusaria may be mentioned. Brandes ('19) found that cultures of *Fusarium cubense*, grown in Richards' solution, when filtered free from the fungus, produced a marked wilting in buckwheat and bean plants, as well as banana leaves. He obtained the same results using Uschinsky's solution as a medium, and concluded that in the disease known as banana wilt, wilting is not due to plugging of the vessels by mycelium, but is probably the result of toxic excretions by the fungus.

Bisby ('19) reports the results of experiments in which leaves

of different plants were placed in filtrates from *Fusarium oxysporum*, *F. discolor sulphureum*, *Fusarium* from pea, and other fungi. Wilting occurred within a few hours with some extracts, a fact which he interprets as not to be explained by changes in acidity. Excised leaves placed in uninoculated media and in water did not wilt until considerable time had elapsed. The injurious effect was also noted after boiling the filtrates and after diluting them considerably. He concludes that the wilting is not due to some poison which is specifically active against certain plants, since potato leaves wilt as readily in old *Rhizopus* solutions as in solutions in which *F. oxysporum* had grown.

Haskell ('19) states that from numerous microscopic observations of the stems of potato plants infected with *Fusarium oxysporum*, he found no instance of a stoppage of the tracheae sufficient to shut off the passage of sap.

In 1920, Young and Bennett ('21) made an effort to determine the method by which *Fusarium oxysporum* induces wilting of potato plants. They found that filtrates of this fungus growing upon Richards' solution were increasingly toxic to potato plants as the age of the cultures increased up to the fortieth day. Furthermore, with this increase in toxicity there was noted, after a 10-day period, a gradual decrease in hydrogen-ion concentration, from an initial reaction of P_H 5.0 to P_H 7.4 on the fortieth day. But when the control (uninoculated) solution was adjusted to P_H 7.4, there was no toxicity noted when cut stems were placed in it. From this it is concluded that the change in reaction, while not the primary cause of wilting, indicated the presence of a compound with a slightly alkaline reaction. Autoclaving or boiling did not alter the toxicity of this substance.

In 1921, Ajrekar and Bal ('21), of India, working on cotton wilt, were unable to obtain wilting of cotton plants by means of the alcoholic extract or by the method described by Hutchinson ('13). They did not, however, conclude from their very meager tests (only 2 plants were used) that no toxins were produced by the fungus; on the contrary, when they sectioned diseased plants and examined them microscopically the number of vessels which were not occupied by the fungus was so great in comparison with

the number occupied that they suspected the action of a toxic substance rather than a mechanical plugging up by fungus mycelium.

Other investigators who have attempted to determine the cause of wilting by various Fusaria or the nature of substances excreted by such parasitic fungi are Picado ('23), Fahmy ('23), Goss ('24), and others.

In the biennial report of the Director of the Kansas Agricultural Experiment Station (page 75) which appeared in 1924, it is recorded that *Fusarium Lycopersici* has been found to secrete an enzyme which, when precipitated, dried and re-dissolved in distilled water, will cause young plants to wilt rapidly when the cut stems are immersed in the solution. The toxicity of the enzyme is destroyed by boiling. Both residue and the dialysate of nutrient solutions upon which the fungus was grown produced wilting of young plants. From this it is concluded that two toxic substances exist, one a colloid and the other a crystalloid.

Quite recently, Rosen ('26) has shown that filtrates representing cultures growing on Richards' solution are quite toxic to cotton plants, while filtrates of cultures growing on media containing organic nitrogen, such as Uschinsky's asparagine solution or peptone-beef broth, are non-toxic. He raises the question if this might not explain why cotton wilt is more prevalent in light sandy soils devoid of appreciable quantities of organic nitrogen than in richer types of soils. By subjecting filtrates of the fungus grown on Richards' solution to distillation *in vacuo*, as well as to ordinary boiling and testing, the distillates and also the residues were found to possess toxic properties, the residue being considerably more toxic. The filtrates of 2- or 3-weeks-old cultures on Richards' solution gave positive tests for nitrites. These were found in quantities ranging from 0.012 mgm. to 0.04 mgm. of nitrogen for each cc. of solution. Using chemically pure sodium nitrite solution comparable to the concentration found in the filtrates, it was determined that this is markedly toxic to cotton plants. Microscopic observations of the vascular elements of wilted cotton plants by Rosen ('26) substantiate the findings of the writer and others that wilting is not due to mechanical plugging up of the vessels by the fungus. Rosen

records that in badly infested fields wilted cotton plants are found occasionally in whose vascular tissues the fungus may be apparently absent, and he suggests that the wilting and interior discoloration of the xylem in these cases is due to the formation of toxic substances by the fungus in the soil. This author concludes that the wilting of cotton infected with *Fusarium vasinfectum* is considered to be due to poisonous chemical substances formed by the fungus, and that filtrates of *Fusarium vasinfectum* growing on Richards' solution possess at least two substances poisonous to cotton. One is a volatile compound with an alkaline reaction and the other is an inorganic salt in the form of nitrite. It is evident from the above references and others not enumerated in this paper that in many of the Fusarium diseases, including those of banana, cotton, potato, and tomato, the theory of mechanical plugging of the fungus as being the factor responsible for wilting is no longer accepted. It is now suggested that toxic substances are probably responsible for wilt symptoms and related pathological phenomena.

EXPERIMENTS RELATING TO TOXIC ACTION BY THE FUNGUS

In order to obtain some information on the probable toxic properties of substances given off by *Fusarium vasinfectum*, the following experiment was performed. The fungus was grown at room temperature for 18 days in 150-cc. Erlenmeyer flasks on 50 cc. of Duggar's nutrient solution previously described, and treated as follows: In a series of 4 flasks, the mats were collected on filter paper by means of a Buchner suction filter, and the filtrates boiled for 10 minutes. Another series of 4 flasks was treated similarly but not boiled. In another series of 4 flasks, the mycelial mats were collected on filter paper as described above, ground with 20 grams of fine quartz sand for 15 minutes in a mortar, and the mixture extracted with 75 cc. of sterile distilled water. Another was prepared in the same way and the extract boiled for 10 minutes. A final series of filtrates was also prepared as described above, and the solution afterward diluted with sterile distilled water at the rate of 1 part filtrate to 3 parts of water. After cutting away the roots, cotton seedlings which had been sprouted in washed sand and which averaged 6 to 8 inches in

height were immersed in 2 of the flasks in each series. In the remaining 2 flasks of each series, the roots of the plants were left intact. Control flasks contained Duggar's nutrient solution and sterile distilled water. All series were placed in the greenhouse in bright light at a temperature of approximately 75–80° C., and examined at intervals of 3, 6½, and 36 hours. The data obtained are presented in table IV.

TABLE IV

EFFECT OF FILTRATES AND MYCELIAL EXTRACTS OF 18-DAY CULTURES OF FUSARIUM VASINFECTUM ON COTTON SEEDLINGS

Series No.	Treatment	Wilt symptoms present after		
		3 hr.	6½ hr.	36 hr.
Filtrate				
1	Boiled	None	Positive	Very pronounced
1 a	Unboiled	None	Positive	Very pronounced
1 b*	Boiled	Slight	Positive	Very pronounced
1 c*	Unboiled	None	None	None
Extract of mycelial mat				
2	Boiled	None	Positive	Very pronounced
2 a	Unboiled	None	None	Positive
2 b*	Boiled	None	Slight	Pronounced
2 c*	Unboiled	None	Slight	Very pronounced
Filtrate†				
3	Boiled	None	None	Slight
3 a	Unboiled	None	Slight	Positive
3 b*	Boiled	None	None	None
3 c*	Unboiled	None	Slight	Positive
Control				
4‡	Boiled	None	None	None
4 a‡	Unboiled	None	None	None
5 §	Boiled	None	None	None
5 a§	Unboiled	None	None	None

* Root removed at base of plants.
† Filtrate diluted 1 to 3.
‡ Duggar's nutrient solution.
§ Sterile distilled water.

From the data recorded, it is observed that both filtrates from the 18-day-old culture of the fungus and an extract made of the mycelial mat of the same age possess toxic properties. In the case of the filtrates, slight evidence of wilting was noticeable

at the end of 3 hours and became quite definite at the end of 6½ and 36 hours. In uninoculated controls of both Duggar's nutrient solution and sterile distilled water the seedlings remained healthy and turgid, even after 36 hours (pl. 32, figs. 2–3). The extracts made from the mycelial mats appear also to possess toxic properties in about the same degree as the filtrates (pl. 32, fig. 2). The seedlings whose roots had been cut away prior to immersing did not reveal wilt symptoms any more pronounced than those in which the roots were left intact. Diluting the filtrates in the ratio of 1 part filtrate to 3 parts of sterile distilled water appears to have lowered the toxicity to some extent, and in these series wilt symptoms became definite only after the maximum period of 36 hours. The toxic substance is approximately thermostable in both the filtrate and mycelial extract used in these experiments. The P_H of the nutrient solution prior to inoculating was 5.6, and at the end of the 18 days the filtrate was 6.8. Additional experiments are now in progress to ascertain if Rosen's ('26) results are correct concerning the presence of nitrites in filtrates of *Fusarium vasinfectum*.

PATHOGENICITY OF FUSARIUM VASINFECTUM ATK.

During the last few years different investigators have raised the question as to the real parasitic nature of the fungus *Fusarium vasinfectum* in connection with wilt of cotton. For instance, Ajrekar and Bal ('21), in their inoculation experiments, do not appear to have secured uniformly successful infection, and they suggested that such negative results perhaps may have been due to the fact that the season was far advanced when their experiments were carried out. They concluded from the result of their inoculation experiments that the Indian species of cotton wilt was not a virulent parasite.

In 1924 Dastur advanced the theory that cotton wilt is not due to the parasitic action of a species of *Fusarium*, but that it may be caused by the excessive accumulation of aluminium and iron salts within the tissues of the plants. He arrived at this conclusion because there was a constant accumulation of iron and aluminium compounds in the tissues of wilting plants and a constant absence of these compounds in the tissues of healthy

plants. He used qualitative microscopical tests for these metals. Tissues of plants which were infected with *Rhizoctonia Solani* Kühn were also examined for aluminium and iron compounds with negative results. Dastur also failed to isolate a parasitic organism from the wilting plants, and suggested that the accumulation of the above compounds may have some correlation with the wilt and that the species of *Fusarium* which had been isolated from wilting plants in different cotton tracts may be only a contributory factor in hastening the death of the plant and merely follows in the wake of the accumulations of these compounds in the tissues.

Dastur worked on varieties and selections of cotton listed as AK_2, AK_4, and Roseum. These were inoculated with different strains of *Fusarium* which had been isolated from wilted cotton plants. He used both virgin soil and sand in pots, and inoculations were made after scraping away the soil an inch or two deep around the plants; and the entire contents of agar tubes, in which the fungus was growing luxuriantly, were placed around the roots. The soil thus scraped away was then returned. In some pots the upper lateral roots were cut before introducing the fungus, but the tap roots were uninjured. His infection experiments were uniformly negative; the controls and inoculated plants were equally healthy and developed good flowers and bolls.

During the progress of this investigation, the writer has endeavored to substantiate the findings of Dastur with reference to the presence of iron and aluminium compounds in the tissues of cotton plants, particularly with plants showing typical wilt symptoms, such as stunted growth, yellowing of leaves, and pronounced vascular discoloration. On August 28, 1926, 3 Trice cotton plants with typical wilt symptoms and 3 healthy plants of the same variety were collected in a field of the Mississippi Experiment Station. These were referred to Dr. W. F. Hand, State Chemist, with a request that quantitative analyses be made for aluminium and iron oxides in the leaves, stems, and roots. By using official quantitative methods, the per cent of iron oxide was determined in the above parts of both healthy and diseased plants, but the results for aluminium were discarded

because of the probable inaccuracy of the present method for quantitative determinations of these compounds in the amounts occurring in cotton plant tissues. The per cent of iron oxide and total ash of the leaves, stems, and roots of healthy and diseased Trice cotton plants are given in table v.

TABLE V

QUANTITIES OF Fe_2O_3 FOUND IN TRICE COTTON PLANTS*

Healthy	Per cent	Diseased	Per cent
Total ash in leaves	14.32	Total ash in leaves	18.54
Iron oxide in leaves	0.028	Iron oxide in leaves	0.04
Total ash in stems	3.75	Total ash in stems	4.11
Iron oxide in stems	0.006	Iron oxide in stems	0.008
Total ash in roots	3.45	Total ash in roots	3.70
Iron oxide in roots	0.016	Iron oxide in roots	0.014
Total ash in plant	21.32	Total ash in plant	26.35
Iron oxide in plant	0.050	Iron oxide in plant	0.062

* Average of 3 plants

Additional Trice cotton plants in a more advanced stage of maturity were collected at the South Mississippi Experiment Station on September 8, and quantitative analyses made for iron oxide in the stems and roots. The results, however, were practically the same as those embodied in table v; i.e., the amount of iron oxide found in both healthy and wilt-infected plants was about the same. The above results do not indicate any close correlation between iron accumulations in the tissues of the plants and wilt infection. The writer has also made numerous qualitative tests for iron with healthy and wilt-infected cotton plants of approximately mature ages. For these tests, potassium acid thiocyanate solution was used, but no appreciable difference could be observed in the stems and roots of healthy and diseased tissues.

In order to test further Dastur's ('24) theory of aluminium and iron accumulations in the tissues of plants as a primary factor involved in producing wilt, another experiment bearing upon this question was planned. In the summer of 1926, a field was visited at the South Mississippi Station which was badly infested with wilt and which had been so for years previous. A quantity of soil from this field was collected and shipped to the laboratory for

some pot experiments. Eighteen pots were filled with the soil and half of them sterilized in the autoclave at 15 pounds pressure for 5 hours. The pots containing the sterile and unsterile soil were then planted with delinted Delfos 6102 cotton seed and placed in benches in the greenhouse, the date being September 15. It was considered that if the disease could be induced in the sterile pots in the absence of the fungus, some significance could be given the probable accumulative effect of certain compounds in the tissues of the plants. The plants were allowed to grow until December 3. Approximate readings were made on November 1, and more accurate readings made by cutting the stems of the plants for evidence of vascular discoloration, on December 3. The results obtained are shown in table VI.

TABLE VI

EXPERIMENTS TO DETERMINE THE PATHOGENICITY OF COTTON WILT, FUSARIUM VASINFECTUM ATK., IN THE GREENHOUSE. SEASON 1926—VARIETY DELFOS 6102

Pot No.	Soil treatment	Number infected plants	
		Apparent wilt Nov. 1	Actual wilt Dec. 3
1	Unsterile	2	3
1A	Sterilized	0	0
2	Unsterile	2	1
2A	Sterilized	0*	0
3	Unsterile	1	2
3A	Sterilized	0†	0
4	Unsterile	2	6
4A	Sterilized	0	0
5	Unsterile	4‡	5
5A	Sterilized	4	0
6	Unsterile	0	1
6A	Sterilized	0	0§
7	Unsterile	8	8
7A	Sterilized	0	0
8	Unsterile	0	8
8A	Sterilized	0	0
9	Unsterile	0	2
9A	Sterilized	0	0

* 2 dead with *Rhizoctonia.*

† 1 dead with *Rhizoctonia.*

‡ 3 killed by *Fusarium vasinfectum.*

§ All infected with *Rhizoctonia.*

The results obtained in the above experiment indicate very clearly that the strain of *Fusarium vasinfectum* present in the soil obtained from the infested field at the South Mississippi

Station was pathogenic and quite virulent, since infection occurred in all of the pots which were not sterilized, and in some cases all of the plants (8 in number) were infected when the plants were taken down and the experiment terminated (pl. 32, fig. 1).

It is quite likely that the failure of Dastur ('24) to secure infection as the result of his inoculations was due to the method he employed. The writer has also frequently found it difficult to infect plants under greenhouse conditions in sand cultures particularly, even after an interval of 90 days, if the fungus is placed merely around the roots of the seedlings. Such a method is not as reliable as that of mixing the fungus thoroughly with the soil or sand prior to sowing the seed. From these and other infection experiments it would seem that cotton wilt cannot be attributed to the accumulation of certain compounds within the tissues, such as aluminium and iron, but that the fungus is responsible for the disease and the attendant symptoms. The recent work of Butler ('26) substantiates this view.

Although the above experiments do not indicate any relation between cotton wilt and the accumulation of iron or aluminium within the tissues of the plants, it is interesting to note the results which Hoffer ('23) has obtained bearing upon this question as it relates to corn root-rot. He has found that in the presence of soluble iron and aluminium salts in the soil or in the presence of other growth-inhibiting factors corn exhibits an abnormal accumulation of these elements in the vascular plate tissues in the nodes of the stalk. There is also some concentration in the leaf tissues. Conditions result which retard the translocation of food to the roots. The roots later cease to function and accordingly their vitality is greatly impaired. He has contributed some very definite data on this point. The most severe cases of root-rot with which he has come in contact were found in soils notable for their deficiencies of lime and phosphate. Potash deficiencies were also often noted. His experiments indicate that root-rot is not due to fungi (as the primary factor), but that these organisms are usually associated with and involved in the crop failure.

In some later experiments ('23), Hoffer and Trost have added materially to the above. A set of acid soils was treated in a manner to increase or decrease the aluminium absorbed by the

plant and to determine the intensity with which soil organisms would attack plants under the conditions. These experiments showed that both lime and phosphate lowered the assimilation of aluminium. The roots of all plants showed rot lesions caused by *Fusarium moniliforme*, but the severity of the damage was modified by the soil treatment and was serious only in the presence of aluminium salts and deficiencies of lime and phosphates. Hoffer also determined the relation of aluminium salts to the susceptibility to root-rot or the invasion by one of the root-rot fungi. In these experiments he used *Gibberella saubinetti*. The results showed that in the presence of aluminium salts there was a predisposition of the plant to invasion by the fungus. A potash reserve in the plant increases the resistance by lessening markedly the accumulations of iron and aluminium in the nodal joints.

In this connection, McGeorge ('25) has also recently found that aluminium is a factor directly associated with the retarded growth of sugar-cane on the acid mauka lands in Hawaii, and further he has found abnormal accumulations of aluminium and iron at the nodes of certain varieties of cane. In the case of these highly acid soils, he considers the retarded growth of the sugar-cane as a direct toxic action of aluminium and not a phosphate deficiency.

ADDITIONAL EXPERIMENTS BEARING UPON ALUMINIUM TOXICITY OF COTTON AND COTTON WILT

Because of the interest manifested regarding the probable absorption of soluble aluminium salts in certain soils and predisposing such plants as corn and perhaps sugar-cane to invasion by soil-inhibiting organisms, it was of interest to study further the rôle that such compounds might play in predisposing cotton plants to infection by *Fusarium vasinfectum*. Accordingly, 2 series of water cultures in battery jars containing aluminium salts in varying concentrations were prepared. Shive's ('16) R_2C_6 nutrient solution was used for this purpose and the aluminium was supplied in the form of the chloride and the sulphate, and added to the nutrient solution to give the following normalities. All treatments were made in duplicate:

SERIES I

	p.p.mil. Al.
1. Control, basic nutrient only	None
2. Basic nutrient plus aluminium sulphate to make N/2500	3.6
3. Basic nutrient plus aluminium sulphate to make N/1000	9
4. Basic nutrient plus aluminium sulphate to make N/500	18
5. Basic nutrient plus aluminium sulphate to make N/250	36

SERIES II

	p.p. mil. Al.
1. Control, basic nutrient only	None
2. Basic nutrient plus aluminium chloride to make N/2500	3.6
3. Basic nutrient plus aluminium chloride to make N/1000	9
4. Basic nutrient plus aluminium chloride to make N/500	18
5. Basic nutrient plus aluminium chloride to make N/250	36

Delfos 6102 cotton seedlings, the seed of which had been previously delinted with sulphuric acid and sprouted in washed sand, were used in these cultures. Twelve seedlings, supported by galvanized wire of a suitable mesh, were placed in each jar when the radicles were about 4 inches long and allowed to grow in the nutrient solutions for 29 days. The solutions were changed in the jars regularly once a week and precaution taken to prevent any contamination with algal forms, etc.

On comparison of the cultures from time to time, toxicity was scarcely apparent in either the series containing the aluminium sulphate or aluminium chloride until a concentration of N/250 (36 p.p. mil. aluminium) was reached. In these cultures, represented in each series by number 5, some toxicity was apparent, not only as related to height of the seedlings, but as to the root development which was also considerably retarded. The effect of these salts when added to the nutrient solution is best indicated by the photographs in pl. 33, figs. 1–2. At the end of the 29-day growth period, the lengths of the tops of the plants were measured and notes made on the condition of the roots. These data are summarized in table VII.

Of the two salts used, the aluminium chloride appears to be slightly more toxic to cotton under the conditions of the above experiment, especially the N/500 and N/250 concentrations.

TABLE VII

RELATIVE LENGTH OF COTTON SEEDLINGS GROWN IN VARYING AMOUNTS OF ALUMINIUM SULPHATE AND ALUMINIUM CHLORIDE FOR 29 DAYS

Culture	Length of tops inches		Condition of roots	
	Sulphate	Chloride	Sulphate	Chloride
Control	11.5	12	Good—normal	Good—normal
N/2500	12.0	10	Good—normal	Good—normal
N/1000	10	9.5	Good—normal	Good—normal
N/500	12	10.5	Good—normal	Fair—few laterals
N/250	8.4	7.8	Fair—few laterals	Poor—very few laterals

After the length of plants in the above experiment had been determined they were put back into the battery jars and to each was added freshly prepared Shive's R_2C_5 nutrient solution. Each culture was then immediately inoculated with a spore suspension of *Fusarium vasinfectum*, in order to ascertain whether the plants which had received varying amounts of aluminium sulphate and chloride would reveal any decided differences to invasion of the fungus as compared with the control plants or the ones which had received the basic nutrient solutions only. At the end of one week after inoculation, all cultures were infected with the fungus (pl. 33, fig. 3) and so far as could be observed, the control plants were equally susceptible to attack by the fungus.

Judging from the above experiment, it seems that aluminium sulphate and aluminium chloride are not appreciably toxic to cotton plants in concentrations lower than 36 parts per million. Moreover, it is believed by the writer, too, that such a concentration of soluble salts of aluminium does not occur in soils of cotton tracts generally in the southern states, and that this factor is perhaps of little consequence in predisposing plants to infection by *Fusarium vasinfectum* as has been supposed by Dastur ('24).

EFFECT OF NUTRITION UPON THE DEVELOPMENT OF COTTON WILT

Greenhouse experiments.—In studying the effects of varying conditions of nutrition upon the development of wilt of cotton, a nutrient solution recommended by Hartwell and Pember ('15) was used as a basic nutrient. This nutrient had the following composition:

.2*N* $Ca(NO_3)_2.4H_2O$	15 cc. per litre
.1*N* NH_4NO_3	10 cc. per litre
.1*N* KCl	8 cc. per litre
.2*N* $MgSO_4.7H_2O$	8 cc. per litre
8.3 grams per litre $CaH_4(PO_4)_2$	1 cc. per litre
$FePO_4$	Trace

Stock solutions of the salts in the above concentrations were prepared in 3-litre bottles and 12 similar bottles were used for the desired dilutions for the different series of treatments.

The series of treatments used were as follows:

Series I—Basic nutrient only.

Series II—Same as I but with phosphorus omitted.

Series III—Same as I but with phosphorus increased 20 times.

Series IV—Same as I but with potassium omitted.

Series V—Same as I but with potassium increased 10 times.

Series VI—Same as I but with potassium increased 20 times.

Series VII—Same as I but containing 1/10 less nitrogen.

Series VIII—Same as I but with nitrogen increased 2 times.

Series IX—Same as I but with calcium omitted.

Series X—Same as I plus 24 p.p.m. iron from ferric ammonium sulphate.

Series XI—Same as I plus 54 p.p.m. iron from ferric ammonium sulphate.

Series XII—Same as I plus 12 p.p.m. aluminium from potassium aluminium sulphate.

Equivalent concentrations of $NaNO_3$, Na_2HPO_4 were used in the above Hartwell and Pember nutrient solution when calcium was omitted from the treatment, and likewise an equivalent of NaCl was employed in the treatments which did not receive potassium.

Three-gallon glazed pots containing washed sand were used for growing the plants, and each series of treatments contained 4 pots. The experiment was begun on August 9, 1926, and on this date each pot of the different series of treatments was planted with delinted seed of Delfos 6102, a staple cotton which is quite susceptible to wilt. After the seedlings were a few days old, they were thinned to 6 plants in each pot. During the first

6 weeks of growth, each series of cultures was given 300 cc. per pot of the nutrient once a week, and after 3 such weekly applications each pot was washed out thoroughly with one gallon of distilled water to prevent the possibility of excess salt accumulation. After 6 weeks had elapsed and the plants became larger they were given 300 cc. of the nutrients per pot every 10 days, and after each pot had received 3 such consecutive 10-day treatments they were washed out with the distilled water as indicated above. On September 9, when the plants were a month old, all series were inoculated with *Fusarium vasinfectum* by applying the entire contents of a bean-agar test-tube culture of the fungus to the roots of the plants. On October 5, all plants in each series were again inoculated by applying to the roots the mat of the fungus which had grown on 50 cc. of Duggar's solution for 10 days. A final inoculation was made on October 20, bits of diseased stems of mature Delfos 6102 cotton plants from a wilt-infested field being placed around the roots of the plants. The schedule of treatments given above was followed as closely as possible, but no attempt was made to keep the pots at a constant soil moisture. Moisture determinations were made, however, with untreated control pots until the plants died of starvation, and these results indicated that the moisture content in the treated pots was approximately 25 per cent of the water-holding capacity of the sand.

Very few of the plants in any of the series of treatments were killed by the wilt, but on March 5, after an interval of almost 7 months, the different series of treatments were terminated and examinations of the plants made as to their green weight, dry weight, vascular discoloration, lateral root discoloration, and the number of plants showing positive infection. Evidence of positively wilt-infected plants was recorded only when such a symptom as pronounced vascular discoloration was obtained by splitting the plants with a stainless steel knife. The numbers with lateral root discoloration were recorded, and undoubtedly, if these plants had been cultured, *Fusarium vasinfectum* could probably have been isolated, though time would not permit such cultural tests for evidence of the fungus. The results obtained in these nutrition experiments are presented in table VIII.

TABLE VIII

EFFECT OF NUTRITION UPON THE DEVELOPMENT OF COTTON WILT IN THE GREENHOUSE—SEASON 1926

Series No.*	Pot No.	Green wt. of plants, gms.			Av. ht. of plants cm.	Dry wt. per series gms.	Vigor	Vascular discoloration			Number wilt-infected plants			Number plants with lateral root discolorations		
		Each	Per series					Each	Per series		Each	Per series		Each	Per series	
			Av.	Total					Av.	Total		Av.	Total		Av.	Total
I	1	50.5			34		Good	0			0			0		
Basic nutrient solution	2	76			34		Good	0			0			0		
	3	58.5			30		Fair	0			0			6		
	4	62	61.5	247	35	79.8	Fair	1†	.25	1	1	.25	1	0	1.5	6
II	1	67			35		Good	0			0			6		
Phosphorus omitted	2	65			31		Fair	4‡			3			4		
	3	64.5			32		Fair	0			0			6		
	4	70	67	266.5	31	82.5	Fair	0	1	4	0	.75	3	0	4	16
III	1	64.6			39		Good	1			1			2		
Phosphorus increased	2	88.5			36		Good	2			2			6		
	3	110			42		Very good	1			1			2		
× 20	4	67	82.5	330.1	33	92.4	Fair	1	1.25	5	1	1.25	5	3	3.25	13
IV	1	87			31		Fair+	1			1			3		
Potassium omitted	2	74			30		Fair	0			0			6		
	3	68.5			34		Fair	1			1			6		
	4	93	85.6	322.5	33	84.9	Fair	3	1.25	5	3	1.25	5	4	4.75	19

TABLE VIII (Continued)

Series No.*	Pot No.	Green wt. of plants, gms.			Av. ht. of plants cm.	Dry wt. per series gms.	Vigor	Vascular discoloration			Number wilt-infected plants			Number plants with lateral root discolorations		
		Each	Per series					Each	Per series		Each	Per series		Each	Per series	
			Av.	Total					Av.	Total		Av.	Total		Av.	Total
V	1	71			33		Fair	1			1			2		
Potassium	2	84.5			34		Fair+	1			1			1		
increased	3	78			31		Good	0			0			0		
× 10	4	68	75.4	301.5	32	78	Good	0	.5	2	0	.5	2	0	.75	3
VI	1	67			33		Good	1			1			0		
Potassium	2	80			32		Good	0			0			0		
increased	3	63			30		Fair	1			1			0		
× 20	4	68	69.5	278	31	73.1	Good	0	.5	2	0	.5	2	1	.25	1
VII	1	88			35		Fair–	3			3			3		
1/10 less	2	67			36		Fair	3			3			4		
nitrogen	3	63			27.5		Fair	2†			3			6		
	4	73	72.7	291	31.5	84.6	Fair	0	2	8	0	2.25	9	0	3.25	13
VIII	1	71			32.5		Fair	2			2			6		
Nitrogen	2	83			38		Good	0			0			0		
increased	3	58			32		Fair	0			0			6		
× 2	4	74	66.5	286	35.5	72.9	Fair	0	.5	2	0	.5	2	0	3	12
IX	1	110			47		Very good	0			0			0		
Calcium	2	114			46		Very good	1			1			1		
omitted	3	130			56		Very good	2			2			2		
	4	131	121	485	53.5	124.1	Very good	0	.75	3	0	.75	3	0	.75	3

TABLE VIII (Continued)

Series No. *	Pot No.	Green wt. of plants, gms.			Av. ht. of plants cm.	Dry wt. per series gms.	Vigor	Vascular discoloration			Number wilt-infected plants			Number plants with lateral root discolorations		
		Each	Per series					Each	Per series		Each	Per series		Each	Per series	
			Av.	Total					Av.	Total		Av.	Total		Av.	Total
X	1	64.5			27		Fair	4			4			3		
24 p.p.	2	72			34		Poor	1			1			4		
m. iron§	3	56.5			29		Poor	5			5			6		
	4	84	69	277	33	71.4	Fair	4	3.5	14	4	3.5	14	6	4.75	19
XI	1	67			27.5		Poor	1			1			6		
54 p.p. m.	2	78			28		Poor	2			2			2		
iron	3	86			30		Fair	6			6			6		
	4	97	82	328	35	77.7	Poor	2	2.75	11	2	2.75	11	6	5	20
XII	1	98			34.5		Fair	0			0			1		
12 p.p. m.	2	100			35.5		Fair	2			2			2		
aluminium‖	3	67			29		Poor	2			2			4		
	4	87	88	352	35	94.2	Fair	3	1.75	7	3	1.75	7	3	2.5	10

* All series contain Hartwell & Pember's basic nutrient solution.

† Slight at base of tap root.

‡ 3 plants with heavy discoloration, 1 plant killed.

§ Iron was supplied from ferric ammonium sulphate.

‖ Aluminium was supplied from potassium aluminium sulphate.

The plants in the pots of each series were photographed on the day the experiment was completed, and they are shown in pls. 34–35.

An examination of the data recorded in table VIII reveals some interesting facts with reference to the nutrition of cotton and the probable rôle it may play in increasing or lowering the resistance of the plant to invasion by *Fusarium vasinfectum*. So far as the writer is aware, no previous attempt has been made to study these questions with respect to cotton and their relation to the development of wilt under greenhouse conditions. It was observed that the disease developed in all of the series of treatments. It was less in series I, which received the basic nutrient without any variation of its nitrogen, potassium, phosphorus, or iron content. As evidenced by the vascular discoloration of stems and tap roots, the infection was highest in series X and XI, containing respectively 24 and 54 parts per million iron from ferric ammonium sulphate. Next in number of wilt-infected plants was series VII, containing 1/10 less nitrogen, series XII, with 12 parts per million aluminium from potassium aluminium sulphate, and series IV, in which potassium was omitted. The pots in which the potassium content was increased were notably free of the disease, as indicated by the lack of vascular discoloration in both stems and roots and the absence of lateral root discoloration. The same is decidedly true of series IX, in which calcium was omitted. In the pots which received marked increases of phosphorus and nitrogen the number of plants with vascular discoloration was low, but the lateral root discoloration was somewhat high.

As revealed by the dry and green weights, the plants in the pots from which calcium was omitted made the most vigorous growth. This was very noticeable as compared with the other series. No toxicity developed in series XII, which received the basic nutrient plus 12 parts per million aluminium salt, and the growth of these plants was good despite the fact that the examination of the roots revealed 7 infected plants and several with lateral root discoloration.

The data as presented do not show any consistent diminution or increase of infections by the various treatments with the exception of the ones receiving increased potassium, the ones from which calcium was omitted, and those in which the iron content

was high. The infection as evidenced by vascular discoloration of stems and roots was low in the pots receiving twice the amount of nitrogen, but, on the other hand, the lateral root discoloration in this series was high, and the fact that the green and dry weight was only a little better than series I (control pots) does not indicate any marked effect as the result of increasing nitrates in the nutrient. The same may be said of increased phosphate in these treatments. Increased potassium content appears to have increased resistance to the fungus, but these plants did not make any better growth, as judged by the dry weight, than did the plants in series IV, in which potassium was omitted. The specific rôle that potash may play in increasing resistance in cotton to invasion by a fungus like that causing wilt is unknown. We know that it is important in the translocation of food materials within plants, and, as Hoffer ('23) suggests in the case of corn, it may also be of importance in the maintenance and normal functioning of the vascular elements of cotton.

On studying the behavior of the plants in the series with high iron content, we find the infection is somewhat high, and this would lend support to Dastur's ('24) theory of iron accumulation as being a predisposing factor to invasion by the fungus. However, this fact is not borne out by quantitative analyses of the tissues of diseased and healthy plants nor by inoculation experiments, as referred to previously in this paper. Because of the pressure of other work, analyses of the ash of the plants in these experiments were not made, but additional nutrition experiments are in progress and this point will receive attention.

The effect of commercial fertilizers upon cotton-wilt infection has also been studied to some extent under greenhouse conditions. On February 3, 1926, duplicate sand cultures were prepared in 10-quart galvanized zinc buckets, each of which had been equipped with special aerating equipment in the form of 2 galvanized zinc tubes attached to the opposite ends of an inverted trough. The sides of the trough supporting the tubes were cut "tooth-like" to facilitate percolation of water when introduced into the buckets. The tubes were also, of course, hollow and when placed in the buckets prior to filling the latter with sand, permitted the plants to be sub-irrigated; at the same

time this facilitated evaporation from the bottom. Approximately 8 kilograms of sand were used in each bucket and the fertilizer was used at the rate of about 800 pounds per acre, regarding 2,000,000 pounds of soil six inches deep as representing approximately one acre. This is in accordance with the usual fertilizer practice in pots when computing on an acre basis.[1] In these experiments, Mississippi Station Trice cotton was used, and the seed delinted with sulphuric acid before planting.

The sand in one of the buckets of each treatment was mixed thoroughly with a bean-agar culture of *Fusarium vasinfectum* and the other left uninoculated as a control. The plants were allowed to grow for 60 days, and then data recorded as to relative height of plants, vigor, and the number infected as evidenced by yellowing of leaves, vascular discoloration, etc. The results are shown in table IX.

TABLE IX

EFFECT OF COMMERCIAL FERTILIZER UPON THE DEVELOPMENT OF COTTON WILT IN THE GREENHOUSE. RATE OF APPLICATION 800 POUNDS PER ACRE. SEASON 1926

Fertilizer formula	Relative height of plants, inches	Vigor	No. plants	Infected plants after 60 days	
				Number	Per cent
8–4–0 check	9.5	Good	8	0	0
8–4–0	10.0	Good	8	3	37.5
8–4–4 check	11.5	Good	8	0	0
8–4–4	10.5	Good	8	0	0
8–0–4 check	9	Good	8	0	0
8–0–4	8.5	Good	8	5	62.5
0–4–4 check	8.5	Fair	8	0	0
0–4–4	8	Fair	8	4	50
12–4–4 check	12	Good	8	0	0
12–4–4	11	Good	8	0	0

From the above results, it appears that potash, when used at the above rate, may have increased the resistance of the plants to infection by the fungus. The results obtained are best indicated in pl. 36, fig. 1.

In other experiments of this nature, the potash in the formula was increased to 6 per cent and the mixture applied at the rate of 600 pounds per acre. However, at the end of 60 days, when the experiment was terminated, no appreciable difference in the

[1] From information supplied the writer by Dr. Oswald Schreiner, of the Bureau of Plant Industry, U. S. Dept. Agr.

amount of infection was noted between plants receiving potash and those to which none was added.

CONTROL

Field experiments.—Field experiments to determine the effects of commercial fertilizers upon the control of wilt were conducted during the season of 1926 at 2 localities in Mississippi. One set of experiments was located at the Central Experiment Station, A. & M. College, and another at the Poplarville Branch Experiment Station. The soil in the experimental plots at the Central Station was artificially inoculated, whereas the field used for recording infection data at the Poplarville Branch Station was already infested with wilt and the plots had been fertilized in the same manner for a period of 5 consecutive years. The size of the plots in the fertilizer treatments at the Central Station was approximately 1/40 of an acre, and at Poplarville approximately 1/12 of an acre.

TABLE X

COTTON WILT OCCURRING IN LONE STAR 65 VARIETY. FERTILIZER TREATMENTS AT CENTRAL STATION. SEASON 1926

Fertilizer formula	Rate per acre lbs.	Yield seed cotton per acre lbs.	Total number plants		% infection		
			in area* inoculated once	in area† inoculated twice	in area inoculated once	in area inoculated twice	Total
Check	——	140	219	42	3.50	5.56	9.06
8–4–8	600	712	288	63	2.20	3.17	5.37
8–4–4	600	572	267	59	3.87	7.35	11.22
8–4–0	600	260	264	61	4.16	5.47	9.63
Check	——	128	258	55	3.49	6.67	10.16
8–8–4	600	684	268	65	3.61	4.30	7.91
8–6–4	600	512	251	54	4.25	6.18	10.43
8–0–4	600	492	227	52	5.00	7.06	12.06
Check	——	140	261	51	3.96	1.98	5.94
6–4–4	600	536	279	59	4.66	8.49	13.15
4–4–4	600	496	296	64	4.28	7.81	12.09
10–5–5	600	708	267	56	3.74	4.80	8.54
Check	——	208	257	55	4.41	4.85	9.26
8–4–4‡	600	652	223	42	4.50	6.76	11.26
8–4–4	900	724	273	53	4.03	7.55	11.58
8–4–4	1200	804	254	54	2.37	2.48	4.85
Check	——	152	226	47	3.70	5.00	8.70

* Soil inoculated May 4 by plowing under re-inoculated sterilized oats.

† Soil inoculated again August 9 by pouring 100 cc. of spore suspension of *Fusarium vasinfectum* around roots of plants.

‡ Lime applied at rate of 4500 pounds per acre, to approximately neutralize soil.

The plots at the Central Station were seeded to Lone Star 65 cotton early in May, and in due time the total number of plants and the percentage of infections were determined. Yield records were obtained in October. These records are given in table x, and represent the average of 3 replications of the one-fortieth-acre plots. The results on infection counts are given in 2 areas in each treatment, one being inoculated just before planting time, the other being a section of the row of each plot which was re-inoculated with 100 cc. of a spore suspension of the fungus in August. The results obtained in a single season at the Central Station on artificially inoculated soil are decidedly inconsistent, and it was only where very heavy rates of fertilizer (1200 pounds per acre) were used that the infection was materially reduced. The addition of lime at the rate of 4500 pounds per acre had no effect in reducing wilt.

The arrangement of plots and the fertilizers used at the Poplarville Branch Station are indicated in table xi.

TABLE XI

ARRANGEMENT OF PLOTS AND AMOUNTS OF FERTILIZERS USED ON COTTON SOIL INFESTED WITH THE WILT FUNGUS, FUSARIUM VASINFECTUM. POPLARVILLE BRANCH STATION. PERIOD OF TREATMENTS 5 YEARS

Plot 3 400 lbs. acid phosphate 150 lbs. ammonium sulphate 50 lbs. potassium sulphate	*Plot 6* 400 lbs. acid phosphate 150 lbs. ammonium sulphate 100 lbs. potassium sulphate	*Plot 9* 150 lbs. ammonium sulphate 100 lbs. potassium sulphate	*Plot 12* 400 lbs. acid phosphate 300 lbs. ammonium sulphate 250 lbs. potassium sulphate
Plot 2 Check	*Plot 5* Check	*Plot 8* Check	*Plot 11* Check
Plot 1 400 lbs. acid phosphate 150 lbs. ammonium sulphate	*Plot 4* 400 lbs. acid phosphate 150 lbs. ammonium sulphate 100 lbs. potassium sulphate	*Plot 7* 200 lbs. acid phosphate 150 lbs. ammonium sulphate 200 lbs. potassium sulphate	*Plot 10* 400 lbs. acid phosphate 100 lbs. potassium sulphate

The plots at the Poplarville Branch Station were planted about the middle of April to Mississippi Station Trice cotton, and on September 2 wilt infection counts made in each plot. The data obtained are presented in table xii.

TABLE XII

OCCURRENCE OF COTTON WILT IN FERTILIZER PLOTS AT POPLARVILLE BRANCH EXPERIMENT STATION. SEASON 1926

Plot No.	Seed cotton per acre, lbs.	Total number plants	Number wilted	Per cent wilted
1	666	493	226	45.84
2	468	622	138	22.19
3	1206	691	102	14.76
4	1278	773	8	1.04
5	648	810	18	2.22
6	1080	762	7	.92
7	1080	660	8	1.21
8	216	612	17	2.78
9	630	665	1	.15
10	486	613	0	0
11	72	512	1	.19
12	990	590	0	0

The results with the above fertilizer treatments indicate that applications of potassium may have been beneficial in reducing the amount of wilt infection. In plot 1, which received adequate phosphate and nitrogen as ammonium sulphate, but no potassium, the infection was even considerably higher than the untreated plots. This is probably explained by the fact that these plants made a very vigorous growth last season owing to the liberal amounts of phosphate and nitrogen used, and such applications may have exhausted the potash reserve in this plot, resulting in a greater prevalence of the disease. At any rate, the writer observed the plants in plot 1 early in July and they were at the time quite large, with wilt beginning to appear over the entire plot. Photographs of the condition of the plants in plots 1, 4, 5, 6, and 12 were made on September 2, also on October 3, when they were maturing. These are shown in pls. 37–39. The spotted areas in plot 1 are especially noticeable, these being due to plants killed early in the season by the wilt organism. It is regrettable that there were not available more plots which had not received potash for a similar period; and until a larger number of such treatments have been made it cannot be definitely stated that the disease may be kept in check by such field fertilizer treatments. A field of Delfos 6102 cotton as shown was photographed by the writer on August 11, 1926 (pl. 38, fig. 2). This field had been planted to cotton for several years and had developed considerable wilt. At the suggestion of the County Agent,

the grower fertilized the crop with 700 pounds per acre of an 8–4–4 fertilizer and left a small portion of the field untreated as a check. Wilt infection counts were made in this field in August and the fertilized portion developed 4.5 per cent wilt, whereas the per cent of infected plants in the unfertilized portion was 26.8 per cent.

It is hoped that the enlarged program of field experimentation which has been inaugurated during the present season of 1927 in Mississippi with reference to the value of fertilizer applications in reducing wilt damage will yield further results.

Field Tests of Wilt Resistance

Tests of wilt resistance have been carried forward for a period of years in cooperation with the Department of Plant Breeding of the Mississippi Experiment Station. These experiments were made on soil which had been artificially inoculated with *Fusarium vasinfectum*. All of the varieties received 600 pounds per acre of an 8–6–4 fertilizer. The total money value per acre of each variety is estimated at twenty-four dollars for cotton seed and average lint prices for middling grade of the indicated length. These lint prices were obtained by averaging quotations of November 1, received from 5 southern market centers. The data obtained in these tests on 19 varieties of cotton for the season of 1926 are summarized in table XIII and were prepared by the Department of Plant Breeding of the Mississippi Experiment Station.

The results indicate that the most promising wilt-resistant staple cottons are Lightning Express, Watson, and Super Seven. Salsbury, another staple cotton, although not included in the experiment in 1926, is somewhat resistant as indicated by experiments made in former years. Rhyne's Cook, Dixie Triumph, Cleveland 54, and Solomon and Oates' Big Boll are promising wilt-tolerant, short-staple varieties. It will be observed that Trice ranked fifth in money value in this experiment. This variety matures its fruit early and rapidly. Frequently, such cottons, even though highly susceptible to wilt, are able to mature a good crop of bolls, especially when the plants are heavily fertilized and the season for growth is favorable.

TABLE XIII

TESTS OF WILT-RESISTANCE AT MISSISSIPPI EXPERIMENT STATION. SEASON 1926

Variety	Pounds per acre		Lint data			Total dollars per acre	Rank in value
	Seed cotton	Lint	Per cent	Length inches	Cents per lb.		
Trice Mississippi Sta.	891	272.6	30.6	1 1/16	13.37	43.87	5
Half & Half, Mahon	534	205.6	38.5	7/8	11.48	27.54	19
Cook, Rhyne	762	287.1	36.5	15/16	12.03	39.27	9
Cook, 307–6	654	228.9	35.0	7/8 f	11.67	31.81	16
Toole, Mathis	741	263.1	35.5	15/16 f	12.37	38.28	11
Toole, Perry	753	255.3	33.9	15/16	12.03	36.68	13
Cleveland, Pied.	903	293.5	32.5	15/16 f	12.37	43.62	6
Cleveland, Humco 20	657	231.9	35.3	15/16	12.03	33.00	15
Cleveland 54	828	278.2	33.6	1	12.70	41.93	7
Super Seven	888	291.3	32.8	1 1/16	14.53	49.49	2
Dixie Triumph	888	293.9	33.1	1	12.70	44.46	4
Kelly Big Boll	660	236.3	35.8	1 f	12.95	35.68	14
Sol. & Oates Big Boll	531	188.0	35.4	1 1/16 f	13.78	30.03	17
Miller	528	178.5	33.8	1 1/16	13.37	28.06	18
D. & P. L. No. 4	669	244.9	36.6	1 1/16	13.37	37.83	12
D. & P. L. No. 5	600	192.0	32.0	1 1/16 f	17.53	38.56	10
Watson	822	259.8	31.6	1 1/16	16.53	49.69	1
Lightning Express	846	256.3	30.3	1 1/16	16.53	49.45	3
Delfos 6102	771	241.3	31.3	1 1/16	14.53	41.42	8

Summary

1. The disease of cotton known as wilt has been further studied and described. An attempt has been made to present a complete discussion of the literature pertaining to the subject.

2. The disease is widely distributed in the Southern States, and the annual loss to the cotton crop in the United States is in excess of 350,000 bales. Such loss varies from year to year, and the amount of it depends somewhat on environmental factors.

3. The disease causes a yellowing of the leaves of the cotton plant accompanied by a stunted appearance of the main stems, usually rather early in the season. Under certain conditions new outbreaks occur at intervals throughout the growing season. The characteristic internal symptom, as in the case of other wilt diseases, is the discoloration of the fibro-vascular tissues of roots, stems, and occasionally leaf petioles. The fungus responsible for the occurrence of the disease is *Fusarium vasinfectum* Atk. Some of the morphological and cultural characters of the fungus are given. The fungus is placed in the recognized generic subdivision "Elegans." This species produces readily (in culture)

micro- and macro-conidia and chlamydospores. The latter are formed in corn-meal or potato-dextrose agar cultures in less than 3 weeks. The color of the stroma is marked and the acid effect well emphasized. The aromatic odor of the fungus when grown on rice is pronounced.

5. Physiological studies of the fungus have been made. These include growth-temperature relations of the fungus in pure cultures, hydrogen-ion relations, and a study of the factors involved in producing wilt. The fungus grows slowly at temperatures below 10° C., the optimum temperatures being 28-30° C., and the maximum temperature about 38° C. It grows well over a rather wide range of hydrogen-ion concentrations. The best growth occurred in cultures represented by P_H values of 3.0, 4.0, 4.5, and 5.5. The maximum growth occurred in cultures started at P_H 3.5. The fungus tolerates a culture solution strongly acid. The growth in the more acid cultures was slow in the beginning, but later more rapid, reaching a maximum growth rate almost as high as in the other series initially less acid. Change in reaction of the culture solution occurred during growth. Cultures originally acid became less so, while those originally alkaline became slightly acid, except the ones started on the extreme alkaline side. As the weight of mat increased, sugar disappeared from the culture solution.

6. The literature on the factors involved in producing wilt in the case of several Fusarium diseases, including cotton wilt, is reviewed, and experiments relating to toxic action induced by the cotton wilt fungus, *Fusarium vasinfectum*, have been performed. Both filtrates and extracts of the mycelial mat of the fungus possess toxic properties. These toxic substances are not destroyed by boiling, but diluting the filtrate lowers its toxicity.

7. Quantitative analyses for iron oxide, in the tissues of mature, healthy, and diseased cotton plants, exhibit no correlation between iron accumulation in the tissues and wilt infection. The pathogenicity of the fungus has been established under certain conditions in the greenhouse.

8. The toxicity of aluminium salts to cotton seedlings is not pronounced, and the susceptibility to invasion by *Fusarium vasinfectum* was no more evident in plants subjected to different

strengths of these salts in a nutrient solution for a certain period than in those grown in the absence of these salts.

9. The effect of nutrition upon the development of the disease in the greenhouse does not indicate any consistent lowering or increase of infection by the various treatments used. Exceptions are noted in the case of certain pot experiments with potassium, calcium, and those receiving additional iron. The most vigorous plants, as evidenced by their green weight, were those in which no additional calcium was added. Increasing the nitrate and phosphate content did not decrease the tendency of these plants to become infected, as evidenced by lateral root discoloration, although the vascular discoloration of the stem revealed by these plants was low. Infection was highest in the series of pots with added iron, but, as previously indicated, quantitative analyses of diseased and healthy tissues do not indicate a correlation between iron oxide content of the tissues and a tendency to become infected when inoculated.

10. Both greenhouse and field experiments have yielded results indicating that potassium salts may be beneficial in reducing wilt damage. Such fertilization methods appear to bring about delayed infection and the plant is able to mature a portion of its bolls, especially if such a system is combined with consistent rotation of crops so as to maintain the organic content of the soil.

11. A study of susceptible and wilt-resistant varieties of cotton has been made, and the behavior of these varieties on soil artificially inoculated with the fungus is described. It is suggested that preference be given to wilt-resistant cottons in tracts where the soil is heavily infested with the fungus. As a further measure of control of the disease, liberal fertilization of the crop is important and preference should be given to fertilizer formulas containing from 8 to 10 per cent phosphoric acid, 4 to 5 per cent potash, and from 4 to 6 per cent nitrogen.

ACKNOWLEDGMENTS

In conclusion, the writer wishes to express his grateful appreciation to Dr. B. M. Duggar, under whose guidance the laboratory and greenhouse aspects of the problem have proceeded, and to thank him for the many suggestions and helpful criticisms which

he has given. He is also indebted to Dr. W. F. Hand, of the Department of Chemistry of the Mississippi A. & M. College, for the services he has rendered on many occasions as to certain chemical phases of the work. Acknowledgments are also due the National Fertilizer Association, who, through their appropriation of funds, made this investigation possible; to Mr. H. H. Wedgworth, of the Mississippi State Plant Board, who kindly made certain of the photographs and recorded wilt data at the Poplarville Branch Station; and to Dr. George T. Moore, for the privileges and facilities of the Missouri Botanical Garden.

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Explanation of Plate

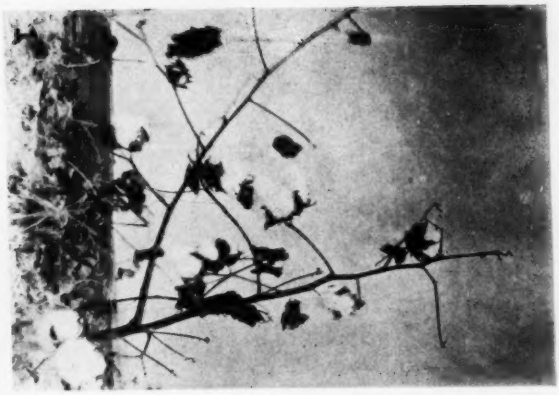
PLATE 31

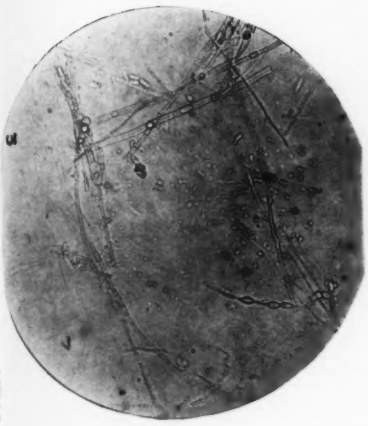
Fig. 1. A cotton plant infected with the wilt fungus, *Fusarium vasinfectum*. Note that the main stem is short and stunted and the lower branch has grown normally.

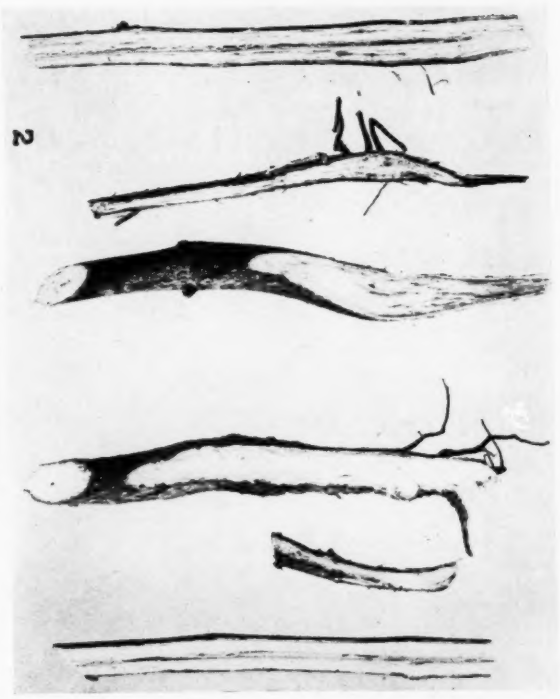
Fig. 2. Stems of mature cotton plants sectioned to show the symptoms of the disease in the vascular elements. At left, three sections of stems from a diseased plant. Note the brown to black discoloration of the fibro-vascular tissues. At right, three sections from a healthy plant.

Fig. 3. Photomicrograph of *Fusarium vasinfectum* taken from a 12-day-old culture of the fungus growing on potato-dextrose agar showing terminal and intercalary formation of chlamydospores. × 100.

Fig. 4. Photomicrograph of *Fusarium vasinfectum* taken from a 10-day-old culture of the fungus growing on potato-dextrose agar showing micro- and macroconidia. × 100.



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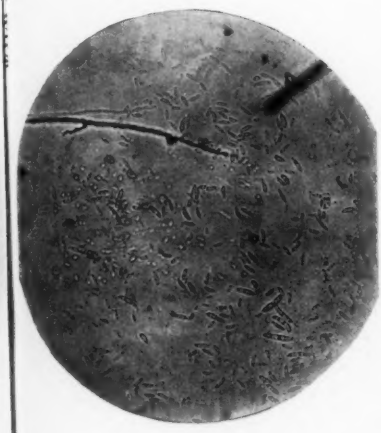


Explanation of Plate

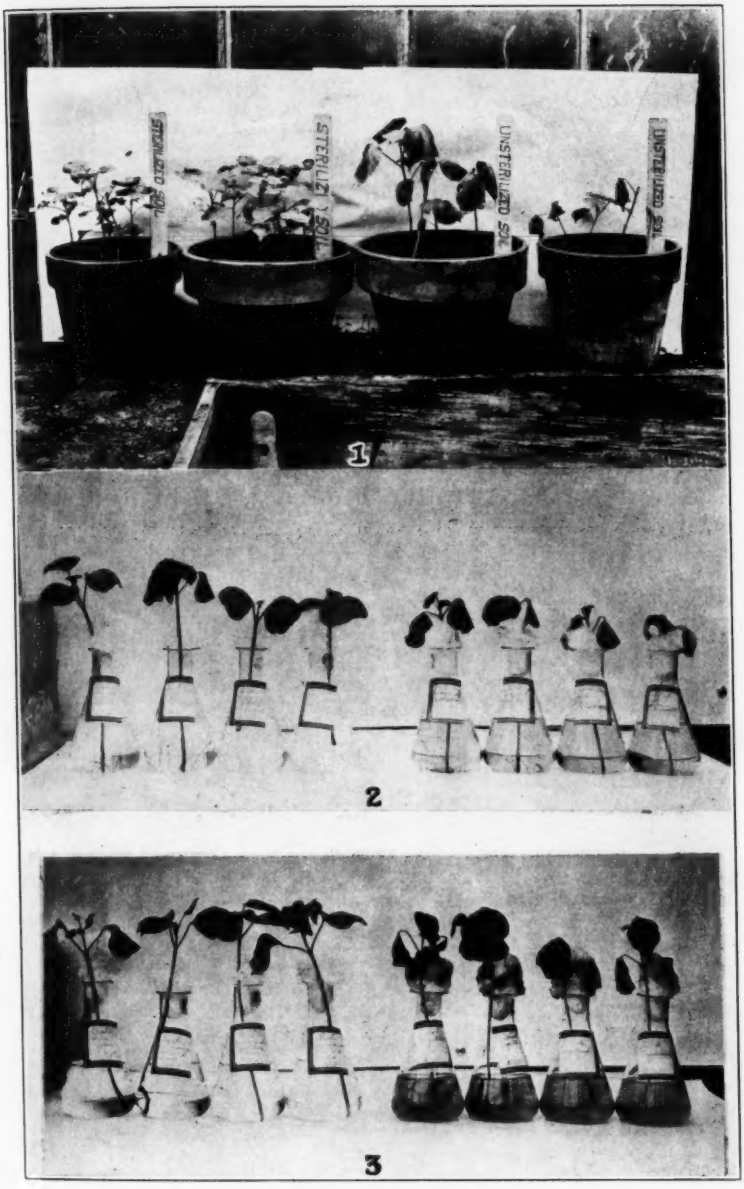
PLATE 32

Fig. 1. Experiments to determine the pathogenicity of cotton wilt, *Fusarium vasinfectum* Atk., in the greenhouse. Season 1926.

The soil in the above pots was heavily infested with the cotton wilt fungus, and was obtained from plot 1 in the cotton fertilizer experiments at the South Mississippi Experiment Station. The soil in the 2 pots at left was sterilized in autoclave for 5 hours prior to sowing seed. The soil in the 2 pots at right was unsterilized. The seed was planted September 15, 1926, and photographed December 3, 1926. Note the wilt in the unsterilized pots. Variety Delfos 6102.

Fig. 2. At left, uninoculated control flasks containing Duggar's solution and cotton seedlings. At right, cotton seedlings in flasks containing filtrates from 18-day cultures of *Fusarium vasinfectum* grown on Duggar's solution. Note toxic effect. All flasks photographed after treatment of 36 hours.

Fig. 3. At left, uninoculated control flasks containing sterile distilled water and cotton seedlings. At right, cotton seedlings in flasks containing extracts of mycelial mats from 18-day cultures of *Fusarium vasinfectum* grown on Duggar's solution. Note toxic effect. All flasks photographed after treatment of 36 hours.



NEAL—COTTON WILT

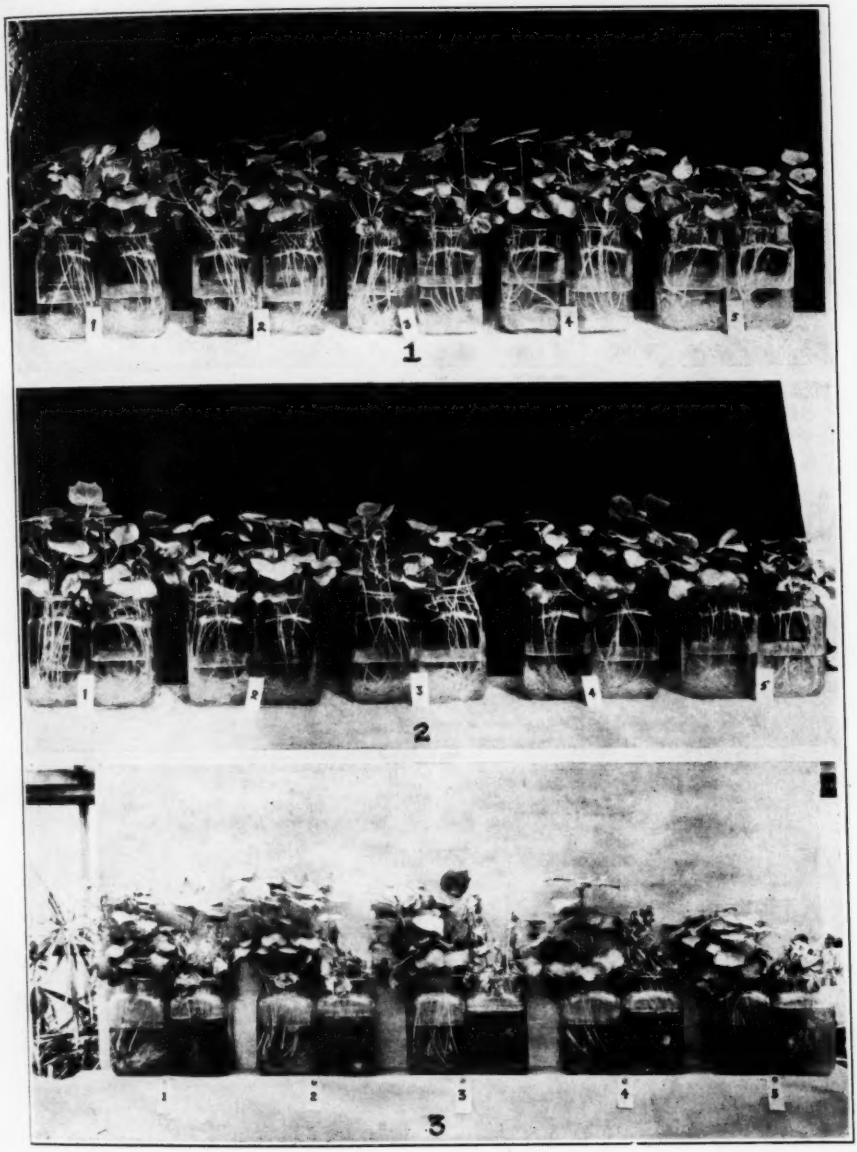
Explanation of Plate

PLATE 33

Fig. 1. Delfos 6102 cotton seedlings grown in Shive's R_4C_5 nutrient solution alone and in this nutrient plus 3.6, 9, 18, and 36 parts per million, respectively, of aluminium, from aluminium sulphate. Slight toxicity is observed in culture 5. Growing period 29 days.

Fig. 2. Delfos 6102 cotton seedlings grown in Shive's R_4C_5 nutrient solution alone and in this nutrient plus 3.6, 9, 18, and 36 parts per million, respectively, of aluminium from aluminium chloride. Note toxicity in culture 5 and retarding effect upon lateral root development. Growing period 29 days.

Fig. 3. Delfos 6102 cotton seedlings grown in Shive's R_2C_5 nutrient solution alone and in this nutrient plus 3.6, 9, 18, and 36 parts per million, respectively, of aluminium, from aluminium chloride for a period of 29 days. All cultures transferred to Shive's R_2C_5 nutrient solution on thirtieth growing day. Seedlings in jars at right of each culture inoculated with *Fusarium vasinfectum* and photographed one week after inoculation. Note that seedlings in all inocula ted jars are infected.



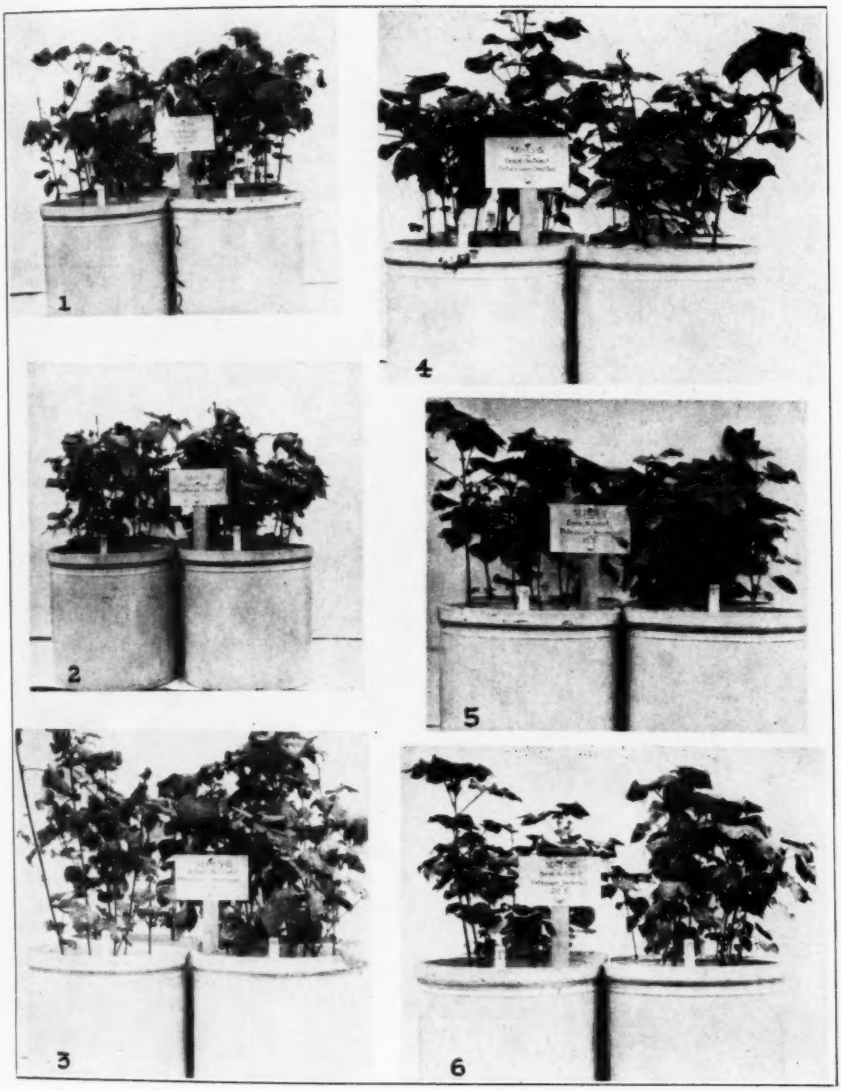
NEAL—COTTON WILT

Explanation of Plate

PLATE 34

Variety of cotton in each series, Delfos 6102. All plants photographed after growing in inoculated sand cultures and receiving the nutrients indicated for approximately 7 months.

Fig. 1. Series I.
Fig. 2. Series II.
Fig. 3. Series III.
Fig. 4. Series IV.
Fig. 5. Series V.
Fig. 6. Series VI.



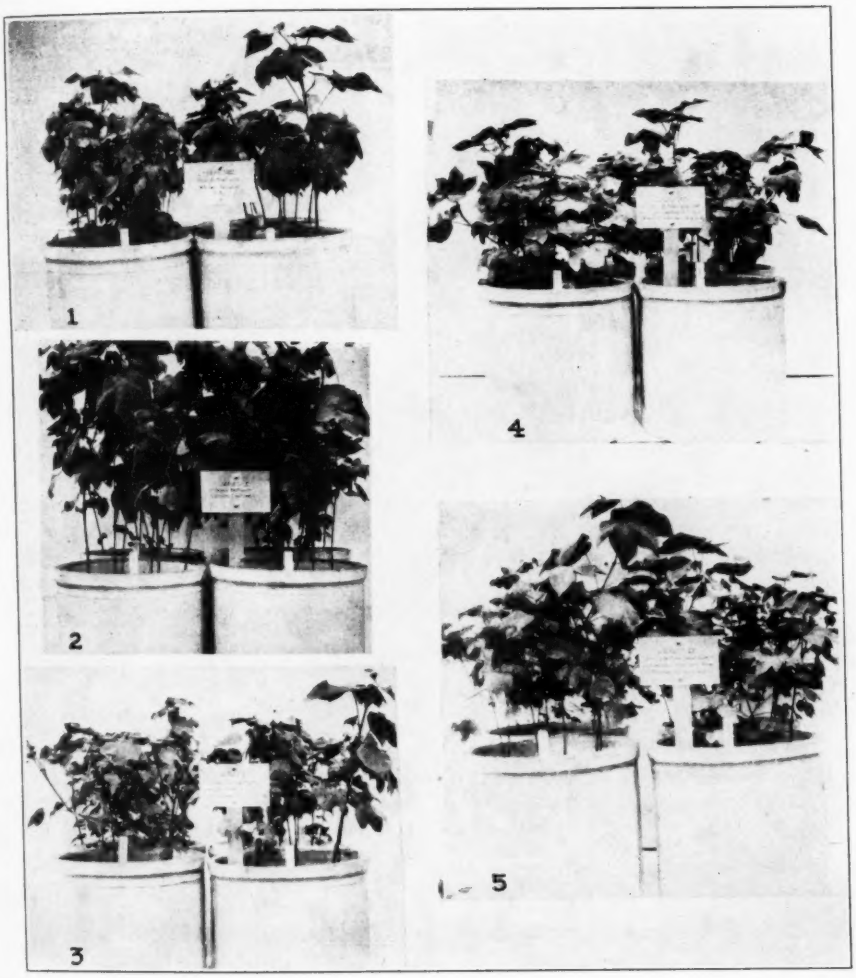
NEAL—COTTON WILT

Explanation of Plate

PLATE 35

Variety of cotton in each series, Delfos 6102. All plants photographed after growing in inoculated sand cultures and receiving nutrients indicated for approximately 7 months.

Fig. 1. Series VIII.
Fig. 2. Series IX.
Fig. 3. Series X.
Fig. 4. Series XI.
Fig. 5. Series XII.



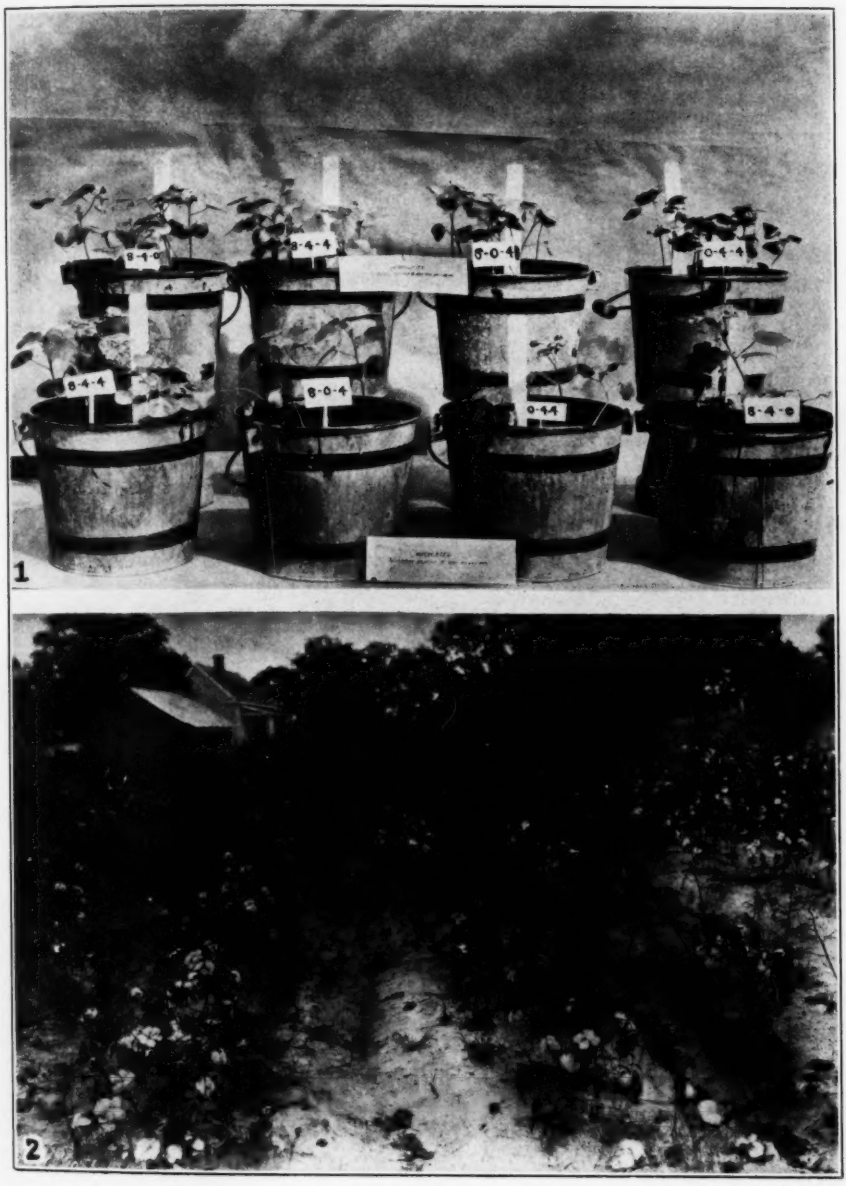
NEAL—COTTON WILT

EXPLANATION OF PLATE

PLATE 36

Fig. 1. Effect of commercial fertilizer upon the development of cotton wilt, *Fusarium vasinfectum*, in the greenhouse. Rate of application 800 pounds per acre. At top, plants uninoculated. At bottom, plants inoculated. Variety, Mississippi Station Trice. Season 1926.

Fig. 2. Cotton plants growing in a field in which fertilization has not been followed. Note the high per cent of wilt. Photographed September 8, at Mayhew, Mississippi. Season 1926.



NEAL—COTTON WILT

Explanation of Plate

PLATE 37

Fig. 1. Cotton fertilizer experiments at South Mississippi Experiment Station, Poplarville. Plot 1, in the foreground, fertilized with 400 pounds acid phosphate and 150 pounds ammonium sulphate. Plot 4, in the background, fertilized with 400 pounds acid phosphate, 150 pounds ammonium sulphate, and 100 pounds potassium sulphate. Note the high per cent of wilt in plot 1. Season 1926.

Fig 2. Cotton fertilizer experiments at South Mississippi Experiment Station, Poplarville. At right, plot 5, check. At left, plot 6, 400 pounds acid phosphate, 150 pounds ammonium sulphate, and 150 pounds potassium sulphate. Season 1926.

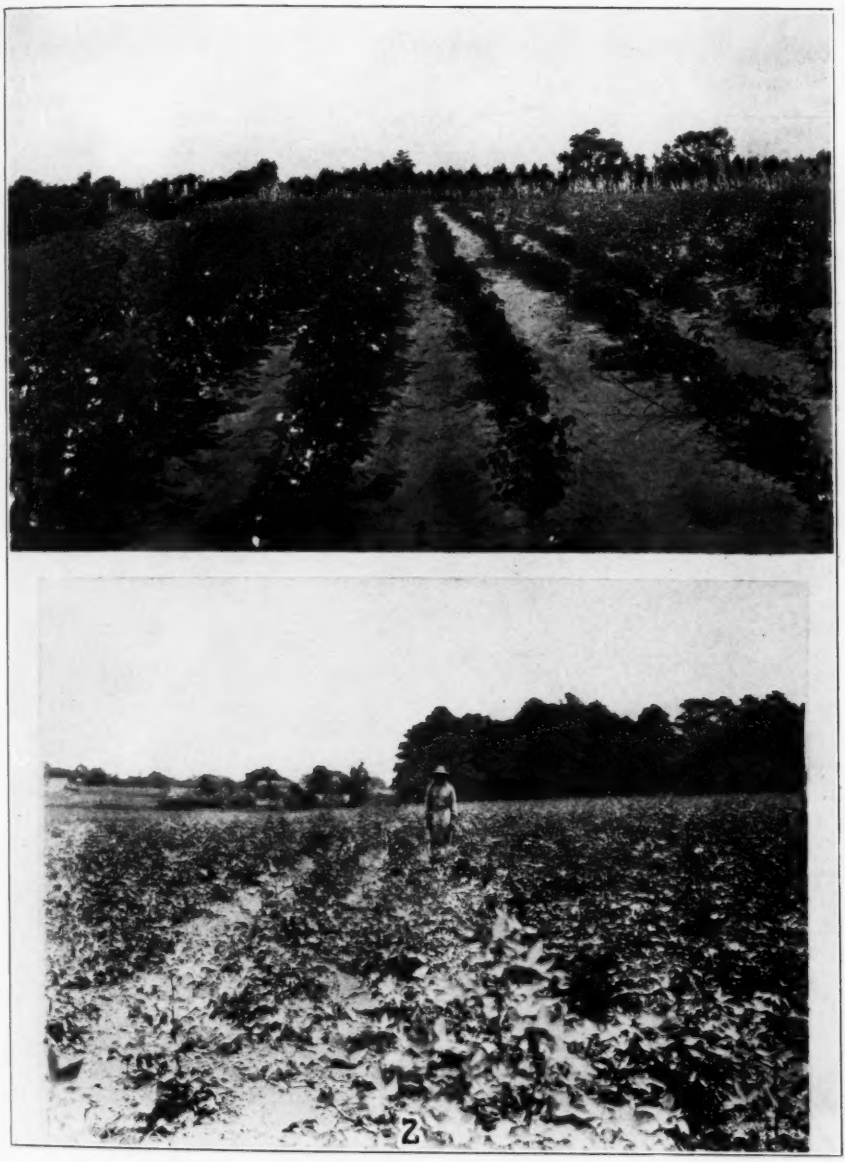
NEAL—COTTON WILT

Explanation of Plate

PLATE 38

Fig. 1. Cotton fertilizer experiments at South Mississippi Experiment Station, Poplarville. At left, plot 12, fertilized with 400 pounds acid phosphate, 300 pounds ammonium sulphate, 250 pounds potassium sulphate. At right, check. Season 1926.

Fig. 2. A view of a cotton field near Aberdeen, Mississippi, showing the effect of a complete fertilizer on wilt control. The rows at left were not fertilized. The rows at right received 700 pounds per acre of an 8–4–4 fertilizer. Season 1926.



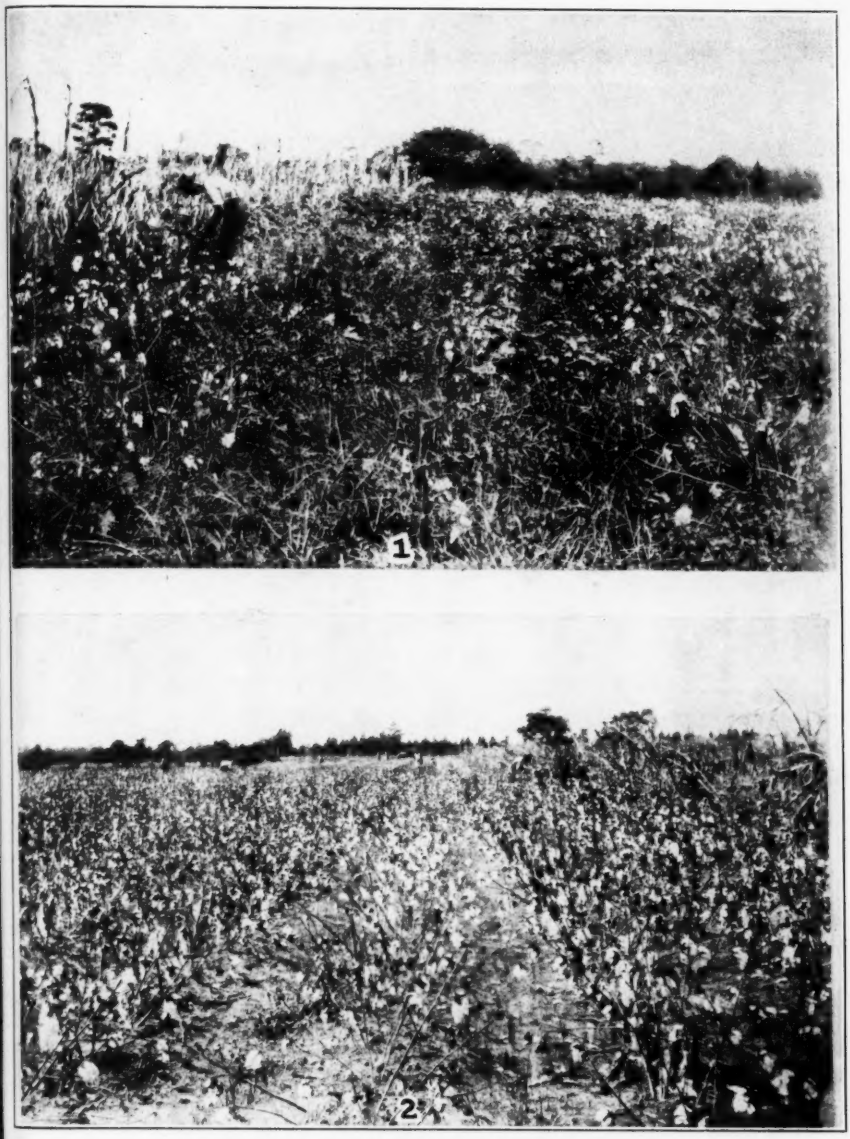
NEAL—COTTON WILT

Explanation of Plate

PLATE 39

Fig. 1. Cotton fertilizer experiments, South Mississippi Experiment Station, Poplarville. Plot 1, fertilized with 400 pounds acid phosphate and 150 pounds ammonium sulphate (referred to in plate 37, but photographed one month later when cotton was mature). Note missing hills and dead plants caused by the wilt fungus. Season 1926.

Fig. 2. Cotton fertilizer experiments, South Mississippi Experiment Station, Poplarville. Plot 4, referred to in plate 37 but photographed one month later when cotton was mature. Fertilized with 400 pounds acid phosphate, 150 pounds ammonium sulphate, and 100 pounds potassium sulphate. Note healthy appearance. Season 1926.



NEAL—COTTON WILT

SPECIES OF CERCOSPORA ON SMILAX IN THE UNITED STATES

L. O. OVERHOLTS

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In the identification of a Missouri *Cercospora* on *Smilax* leaves it was found desirable to examine rather critically authentic material of the different species reported on that host. These species are as follows: *C. Smilacis* de Thuem; *C. Smilacina* Sacc.; *C. Petersii* (Berk. & Curt.) Atk.; *C. mississippiensis* Tracy & Earle; *C. subsanguinea* Ell. & Ev.; and *C. nubilosa* Ell. & Ev. Of these, *C. nubilosa* can be left out of the subsequent discussion, since an examination of the type collection in the herbarium of the Missouri Botanical Garden shows that it is on leaves of *Dioscorea villosa* L. and not on *Smilax* as originally reported. On the original label the host is recorded as "Smilax ?," but the doubt indicated by the question mark was not incorporated in the record when the species was described. However, comparisons of the leaves with those of *Dioscorea villosa* L. shows conclusively that they are from plants of the latter species.

C. subsanguinea has been available only from one collection, on *Maianthemum canadense*, distributed in 'Fungi Columbiana,' No. 4110. On this host it forms irregular necrotic areas 1 cm. or more broad and long, not bordered by a distinct margin. This is in sharp contrast to the type of spot produced by the other species on *Smilax*. The fasciculate conidiophores are abundant on the lower side of the leaf over all the dead area. They measure 40–240 × 6 μ. The spores also are distinctive, being practically cylindric, 10-celled or more when mature, and measure 45–120 × 5–6 μ. If these are the characters of that species when on *Smilax* as well, it need not be confused with the species discussed below.

The other collections examined, numbering in all about thirty, readily fall into two groups on the basis of size and shape of the spores. Before describing these in detail it may be well to state that an examination of this series of collections has demonstrated

conclusively that one can easily be misled by failing to recognize the variability in the shape and coloration of the conidiophores in young and old specimens. Probably in all species of *Cercospora* the conidiophores are at first straight and pale-colored (pl. 41, fig. 4), i.e., pale fuscous or pale cinnamon. The conidia originate apically, and one may find an entire collection in which the conidiophores and the conidia are in this stage of development. If the fungus continues growth, however, the apical conidium is pushed aside as the conidiophore grows distally from a point near the point of origin of the first conidium. This manner of growth forms an offset in the conidiophore hypha at that point. Another conidium is produced on the apex of the new growth, and it in turn may be pushed aside and another offset results. As a result of this method of growth an old conidiophore may become very irregular at its apex. At times the direction of growth assumed by the conidiophore after the production of one conidium may be almost at right angles to that of its previous growth, in consequence of which the mature conidiophore may present a sharp elbow near its distal end (pl. 41, fig. 7). Likewise the color of the conidiophore becomes darker with age, so that while in a young condition it may appear rather pale, in an older stage it assumes a darker color, in the species discussed here becoming dark reddish-brown or chestnut on the lower part, the apex remaining somewhat paler.

Length of the conidiophores is also a factor on which little reliance can be placed in the genus if the conditions in the species on *Smilax* can be taken as a criterion. For example, in a collection distributed by Nash in 'Plants of Florida,' No. 1872, the conidiophores measure 40–75 μ long, and essentially the same measurements are obtained from de Thuemen, 'Myc. Univ.' No. 1670. However, in Nash, 'Plants of Florida,' No. 1893, they measure 45–135 μ and in a collection by Peck at Manor, L. I. (Herb. N. Y. State Mus.) they measure 72–225 μ. Yet in other characters these three collections are so similar that there can be no doubt they should be referred to the same species. Likewise a collection by Peck at Wading River, N. Y., gives conidiophores 50–140 μ, while one at Arcadia, Mo. (Overholts Herb. 10426) has them 75–210 μ; in 'Plants of the Gulf States,' No. 7802, they

are 150–180 μ long; and in Nash, 'Plants of Florida,' No. 2125, they are 105–165 μ. Moreover, I have found that mounts from different spots on the same leaf give about as much variation as may be exhibited by different collections, so that it has become quite evident that, in the species on *Smilax* at least, little reliance can be placed on the length of the conidiophores.

After examining all the available collections it is apparent, as stated above, that they readily fall into two categories on the shape and size of the spores, so that of the four described species yet to be considered, two only can remain as valid. One of these is *C. Smilacis* de Thuemen, in which the conidia are subcylindric, tapering very gradually to a narrowed apex, and hence in reality slightly obclavate when seen on the conidiophores. Frequently they are considerably curved. They measure for the most part 60–135 × 4–5 μ, but some as short as 40–50 μ are to be found, and perhaps longer ones also could be located. The septations are most frequently only two or three in number but may vary to ten or eleven, or perhaps more (see pl. 40).

The other collections must be referred to *C. Smilacina*. American specimens were first so identified by Saccardo, the material being the same collection that de Thuemen had apparently referred for Peck to his own *C. Smilacis*. Since Peck reported it as this species (Ann. Rept. N. Y. State Mus. **33**: 29. 1880) it is sometimes cited in synonymy as *C. Smilacis* Peck. The species differs from *C. Smilacis* in the conidia that are usually rather abruptly narrowed at one end and have a tendency to be snow-shoe-shaped or the shape of a tailed pumpkin seed. Most of the conidia measure 40–65 × 5–6 μ, but a few are as short as 30 μ and 4–5 μ in diameter, while occasionally a spore as much as 75 μ long has been seen. They may be as much as 8-celled, but usually show less than that number. In general, therefore, they are much shorter than those of *C. Smilacis* and are more sharply contracted at one end (see pl. 41).

How often it occurs that *Cercospora* conidia are clavate and not obclavate on the conidiophores, remains for further study to determine. As originally founded by Fresenius ('Beitrage zur Mykologie,' p. 91, 1863) the genus is based on *C. Apii*, which he illustrates as with obclavate conidia, and the usual conception

of the genus involves that characteristic. While I have not seen a large number of clavate spores on conidiophores in *C. Smilacina*, yet they have been observed several times, though always obviously immature, and at a later stage of maturity they may possibly take a more obclavate form.

I find nothing in the character of the spots produced by these two species to aid in their macroscopic separation. They vary to a considerable extent on the different hosts, with variation in thickness and texture of the leaves. Always the spots produced by both species are subcircular in form and at first dark in color, the center usually becoming lighter in color with age, leaving a narrow, dark red or purplish, often raised margin. Yet in some collections the margin is not well marked. The conidiophores are usually hypophyllous in both species, but at times are amphigenous, though this tendency to occur on both surfaces seems more marked in *C. Smilacis* than in *C. Smilacina*.

A few words may be said regarding *C. Petersii*. The species was originally described as a *Helminthosporium* (Grevillea **3**: 102. 1875), but was transferred to *Cercospora* by Atkinson (Jour. Elisha Mitchell Scientif. Soc. **8**: 25. 1892). I have examined mounts from the type collection of this species but failed to find the conidia and found only a few conidiophores, and was not able to arrive at any decision as to the exact status of the species. In the sense of Atkinson (*l.c.*) and as determined in several collections by Ellis it most certainly is *C. Smilacina*, and I am of the opinion that it must be regarded as a synonym of that name. It is recorded in Saccardo's host index as on the stems of *Smilax*, evidently an error, since the original description specifically locates it on the lower surface of the leaves of *Smilax* and of *Laurus*.

The following diagnoses of the two species most concerned in this treatise are appended:

Cercospora Smilacis de Thuemen, Myc. Univ. No. 1670. 1880. Pl. 40.

C. mississippiensis Tracy & Earle, Bull. Torr. Bot. Club **22**: 179. 1885.

Spots subcircular, usually limited by the veins, 1–7 mm. in diameter, at first dark purplish red, then becoming paler at the

center while retaining a dark conspicuous margin; conidiophores fasciculate, hypophyllous or amphigenous, septate, pale cinnamon to fuscous or chestnut, at first straight and paler at the tips, in age becoming chestnut color and the tips nodulose or wavy, 45–225 μ long, 3–5 μ broad; conidia subcylindric, gradually narrowed at the apex, hence somewhat obclavate, 3–4-celled or becoming 10- to 14-celled, pale fuscous, 60–135 × 4–5 μ.

On living leaves of various species of *Smilax*.

The species differs from *C. Smilacina* Sacc. in the longer spores that are sub-cylindric and not sharply narrowed.

Specimens examined: de Thuemen, Myc. Univ. Nos. 1670 (type) and 1768; Starkville, Miss., 1891, *Tracy* (type of *C. mississippiensis* Tracy & Earle) (Mo. Bot. Gard. Herb. and Herb. N. Y. State Mus.); Manor, L.I., *Peck* (Herb. N. Y. State Mus.); Columbus, Miss., 1895, *Tracy* (Mo. Bot. Gard. Herb.); Nash, Plants of Florida, Nos. 1893, 1872, and 2073 (Mo. Bot. Gard. Herb.); Ellis, N. Am. Fungi, No. 1251 (Mo. Bot. Gard. Herb.); Bairds, Miss., *Tracy* (Mo. Bot. Gard. Herb.).

Cercospora Smilacina Sacc. Michelia **2**: 364. 1881. Pl. 41.

? C. Petersii (Berk. & Curt.) Atk. Jour. Elisha Mitchell Scientif. Soc. **8**: 25. 1892.

? Helminthosporium Petersii Berk. & Curt. Grevillea **3**: 102. 1875.

Spots subcircular, usually limited by the veins, 1.5–5 mm. in diameter, at first uniform dark red or purplish black, becoming paler at the center as the spot expands, retaining (usually) a dark, somewhat raised border; conidiophores hypophyllous or rarely amphigenous, fasciculate, septate, pale cinnamon to chestnut below, usually paler above and there quite wavy or nodulose in extreme age, sometimes bent almost at right angles near the upper end, 50–215 μ long; conidia terminal and lateral, rather abruptly narrowed toward one end, pale fuscous to brownish, usually 2- to 4-celled, sometimes 6- to 8-celled, 40–65(–75) × 6 μ.

On living leaves of various species of *Smilax*.

The species is easily separated from *C. Smilacis* by the conidia that are rather abruptly narrowed near the middle, and less than 75 μ long.

Specimens examined: Wading River, N. Y., 1879, *Peck* (Herb. N. Y. State Mus.); Shelter Island, N. Y., *Clinton* (Herb. N. Y. State Mus.); Plainville, Conn., 1883, *A. B. Seymour* (Mo. Bot. Gard. Herb.); Ravenel, Fungi Am. Nos. 166 and 616 (Mo. Bot. Gard. Herb.); Hume, Florida Fungi, No. 62 (Mo. Bot. Gard. Herb. and Herb. N. Y. State Mus.); Tracy, Plants of the Gulf States, No. 7802, Auburn, Ala., 1897 (Mo. Bot. Gard. Herb.); Auburn, Ala., 1896, *Underwood* (Mo. Bot. Gard. Herb.); Nash, Plants of Florida, No. 2125 (Mo. Bot. Gard. Herb.); Seymour & Earle, Economic Fungi, No. 199 (Mo. Bot. Gard. Herb.); Bartholomew, Fungi Col. No. 2808 (Mo. Bot. Gard. Herb.); Arcadia, Mo., 1926 (Overholts Herb., No. 10426, and Mo. Bot. Gard. Herb.).

Explanation of Plate

PLATE 40

Cercospora Smilacis

Fig. 1. Conidia from de Thuemen, Myc. Univ. 1768. × 400.

Fig. 2. Fascicle of conidiophores emerging through a stoma. Ellis, N. Am. Fungi, 1251. × 160.

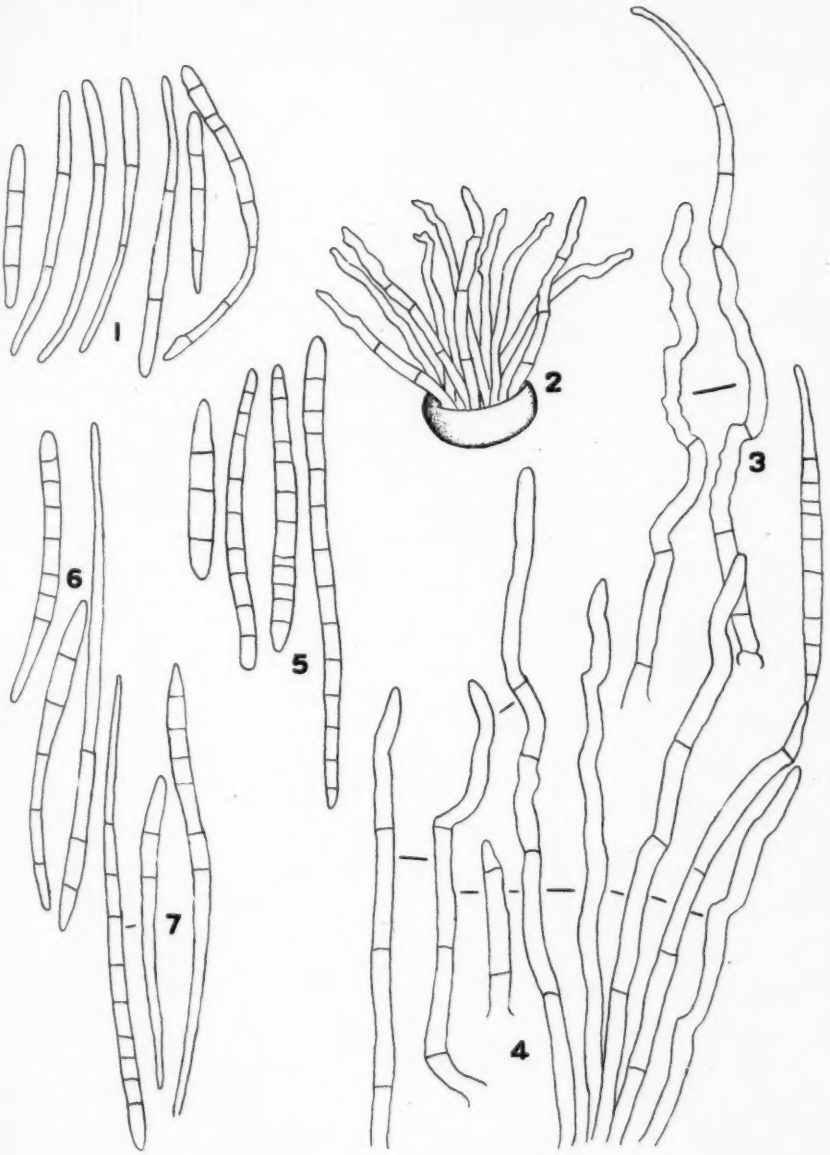
Fig. 3. Conidiophores, one bearing an obclavate conidium. From de Thuemen, Myc. Univ. 1768. × 400.

Fig. 4. Conidiophores, one bearing a conidium. From deThuemen, Myc. Univ. 1670. × 400.

Fig. 5. Conidia. From Ellis, N. Am. Fungi, No. 1251. × 400.

Fig. 6. Conidia. From the type collection of *C. mississippiensis*. × 400.

Fig. 7. Conidia. From collection at Starkville, Miss., by Tracy, 1892. × 400.



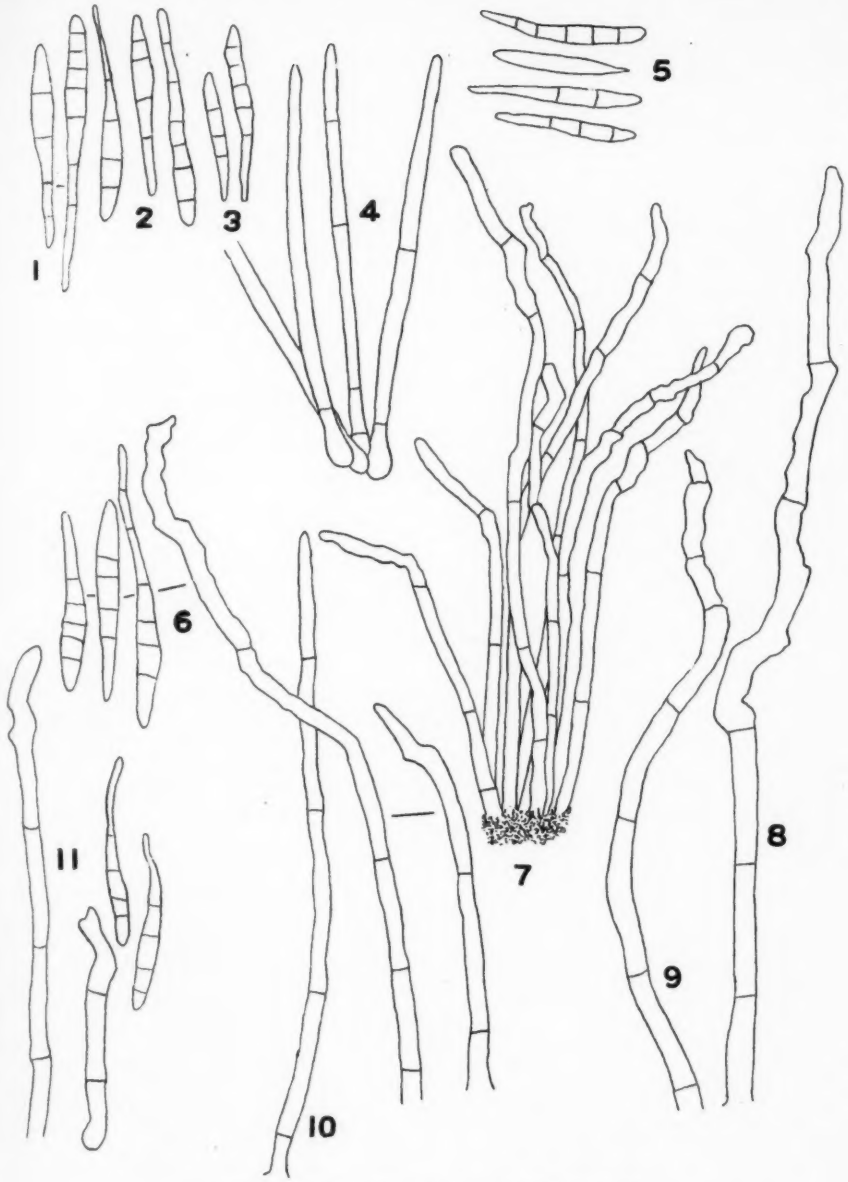
OVERHOLTS—CERCOSPORA ON SMILAX

Explanation of Plate

PLATE 41

Cercospora Smilacina

Fig. 1. Conidia. From Tracy, Plants of the Gulf States, No. 7802. × 420.
Fig. 2. Conidia. From collection at Arcadia, Mo., 1926, Overholts Herb. 10426. × 420.
Fig. 3. Conidia. From Seymour & Earle, Economic Fungi, No. 199. × 420.
Fig. 4. Conidiophores in young condition. From collection at Auburn, Ala., 1896. × 420.
Fig. 5. Conidia. Same as fig. 4.
Fig. 6. Conidia and two conidiophores. From Bartholomew, Fungi Col. 2808. × 420.
Fig. 7. Fascicle of conidiophores. From collection at Arcadia, Mo., 1926, Overholts Herb. 10426. × 165.
Fig. 8. Old conidiophore. Same as fig. 7. × 420.
Fig. 9. Old conidiophore. From Seymour & Earle, Economic Fungi, No. 199. × 420.
Fig. 10. Conidiophore. From Tracy, Plants of the Gulf States, No. 7802. × 420.
Fig. 11. Two conidiophores and two conidia. From collection at Wading River, N .Y., Peck, 1879. × 420.



OVERHOLTS—CERCOSPORA ON SMILAX

GENERAL INDEX TO VOLUME XIV

New scientific names of plants and the final members of new combinations are printed in **bold face** type; synonyms and page numbers having reference to figures and plates, in *italics;* and previously published scientific names and all other matter, in ordinary type.